

# Adam Smith is too long dead

The United States and the EEC have made some progress towards an understanding that their agricultural subsidies will eventually be phased out, but the pace of change is far too slow.

WHEN the French were organizing their revolution 200 years ago, the British were toying with a less spectacular but (as events showed) more enduring idea: that commerce, the buying and selling of goods and services, is the best mechanism for making economic systems efficient. Adam Smith's idea, which eventually led the British to repeal the Corn Laws which kept foreign grain away, is simply that a society can expect to become more prosperous if its members concentrate at work on the tasks they perform most efficiently ("division of labour" is the catchphrase). A precondition is that people should be free to satisfy all their needs by making purchases in the market, and that they should not be exploited in doing so by sellers which are, for example, monopolies. The trade, in other words, must be free. All that, of course, while the foundation of nineteenth-century liberalism and the basis of anti-trust legislation in the United States and elsewhere, is now kids' stuff. Yet, sadly, it is in danger of being forgotten by people who should know better.

Most easily forgotten because the evidence is so familiar is the simple truth that the increase of the prosperity of the rich countries of the world since the Second World War is largely the result of the international extension of Adam Smith's doctrine. The evidence for that is clear. For 40 years, the volume of international trade has increased, year by year, faster than the wealth of any single country, Japan not excepted. The process has been assisted by a bargain struck between the rich countries — the General Agreement on Tariffs and Trade (GATT) — in which they have undertaken not to discriminate among their potential trading partners in respect of certain goods and services. A country may still levy tariffs on imported goods, but must do so equitably, without regard to their origin. Winning extra exports by subsidizing them is against the rules. But GATT, as it stands, is not sufficient to ensure an efficient distribution of labour between the rich countries, let alone to extend its benefits to the poor countries. It is merely a step in that direction.

That is why GATT includes provisions for its own periodical revision. Just such a process has been under way for the past two years. The hope is that GATT, now chiefly concerned with manufactured goods, can be extended to cover trade in agricultural products and some kinds of financial services. The agricultural issue is especially important: as things are, the United States and the members of the EEC each spend roughly \$30,000

million a year supporting their farmers, with the result that, in Europe, the cost to consumers of some basic foodstuffs is several times the world market price. But the self-inflicted damage done by this protection goes far deeper; both regions would be even more prosperous if the people induced to work as farmers were instead encouraged to work at tasks that add more value to what they produce. Yet agriculture is precisely an activity in which other countries can realistically hope to become major suppliers. A group of them, called the Cairns group, which includes Australia and many South American countries, has been correctly making just this point for several years.

Sadly, the GATT meeting at Geneva two weeks ago was again inconclusive. The United States and EEC have agreed between themselves that they will eventually eliminate agricultural subsidies, but the timetable and even the mechanism are still unspecified. The best hope is that there will be some kind of timetable two years from now and that the distortions in agricultural trade will be unwound over the succeeding decade. How complete the process will be is far from clear.

That is too long a time to wait for two quite different reasons. First, tangible benefits will be needlessly postponed, as will be their sharing with the really poor countries of the world. And the mere passage of time must increase the danger that GATT itself will be undermined. Both in the United States and Europe, consumers of manufactured goods are already paying needlessly high prices because of the creeping incidence of protectionism, mostly directed against Japan. GATT could be a tattered treaty when the time comes for its formal revision.

That is why the United States and the EEC, the chief players for the time being in the detailed GATT negotiations, should steel themselves for some unpalatable truths buried not very deeply in Adam Smith's old doctrine. During the past few years, both economic groups have sought to restrict imports of products as different as motor-cars and dot-matrix printers from Japan on the grounds that Japanese manufacturers are selling their products "too" cheaply and in the belief that continuing imports will damage their own economies. But the conclusion could be the opposite of the truth. Whether exports of these products are subsidized or not, if their prices are less than occidental manufacturers must charge, they are bargains that should be snapped up.



Exceptionally, there may be strategic arguments that weigh in the other direction. The fear that imports will rob domestic economies of jobs is relevant only if nobody can think how those who may be displaced might be better employed. It is of no concern, in the calculation of whether a Japanese dot-matrix printer is a bargain or a threat to economic survival, to ask whether Japan will "reciprocate" by buying US beef or Scottish whisky (two other fashionable bones of contention). The lesson is that free trade is worthwhile even if the policy is unilaterally applied. If that were more generally understood, there would be a better chance that the half-way house called GATT would survive the enforced changes it now suffers.

## Himalayan hoax

The palaeontology of the Himalayas has been corrupted by an apparently systematic misplacing of fossils.

PALAEONTOLOGY is renowned for spectacular deceptions, among which 'Piltdown man' is generally the best-known, but the trouble caused in the Himalayas by the activities of Professor Viswa Jit Gupta of the Punjab University of Chandigarh will cast a longer shadow, as Dr John Talent shows (see page 613). For there was only one Piltdown skull which many, from the outset, believed to be a hoax. Gupta's cumulative joke, going back over a quarter of a century, will be excised from the record only with much greater difficulty.

Talent tells an extraordinary tale. In one series of published papers, specimens from a well-known and apparently unique conodont fauna recovered from a quarry in New York State were reported to have been found in a quartzite formation spanning north-east India and Nepal. Ammonoids with all the characteristics of specimens well-known from Morocco were similarly reported from nearby in the same formation, implying that biostratigraphical time must at some stage have stood still. On at least one occasion, the same specimens were described as having been found in two places. The general effect of these misplaced fossil finds is to argue for a Late Silurian or Devonian rather than Early Silurian age for the Muth quartzite.

Palaeontologists now have two tasks: to clean up the mess and to prevent it happening again. Sadly, it will not suffice simply to discard all the information in Gupta's published papers, estimated to number more than 300. Who can tell how many other studies have been falsely founded on his disinformation? Moreover, there is little chance of being able to check the accuracy of the reports in the field; the region is not easily accessible, while the locations from which fossils have been reported are inadequately defined. The now improved understanding of the Himalayan region provided by plate tectonics (which may have helped to throw light on the inconsistencies suggested by Gupta's work) may simplify the task of dating rock formations in the region, but that will put the

cart before the horse, denying biostratigraphy the opportunity to test the theory of plate tectonics.

Nor will prevention be easy. To the extent that many of Gupta's misplaced specimens appear to have come from teaching collections, some enterprising group of palaeontologists might think of authenticating indelibly marked specimens for the commercial trade, hoping that unmarked fossils would then become less desirable. But that is a mere technical fix. Journals have a part to play, especially in insisting on adequate locations of fossil finds. But the only durable remedy is to create an intellectual climate in which people's willingness to hazard colleagues' trust is diminished to zero. (To that end, palaeontologists should, on this occasion, have been quicker to voice their doubts.) The rewards of a copious bibliography are still excessive. □

## Cold fusion in print

The appearance next week of one, not two, papers on cold fusion should not be misunderstood.

NEXT week's issue will include an article by Dr S.E. Jones and his colleagues at Brigham Young University and the University of Arizona on which the group has based its claim to have observed nuclear fusion in an electrolytic cell, but next week's issue will not include the article by M. Fleischmann (Southampton) and S. Pons (Utah) which the authors have said publicly that they had submitted for publication. That requires an explanation.

The articles were received on 23 and 27 March respectively and, following standard practice, were sent to referees. In each case, several questions requiring clarification and even amendment of the texts were raised. Jones *et al.* have been able to amend their text in a way that satisfies the referees, but Fleischmann and Pons have taken the view that they could not at the same time satisfy the referees and get on with other urgent work. No doubt a relevant consideration was the early publication in the *Journal of Electroanalytical Chemistry* of an extended version of the article sent to *Nature*.

It is important that these events should not be misunderstood. It is entirely within the gift of authors to decide whether it is worthwhile to reply to referees' comments. The peer-review process is not, after all, a court of law (and should not be mistaken for such). It follows that the non-appearance of the article must not be taken to imply that the experiments described elsewhere by Fleischmann and Pons are inherently less believable than those of Jones *et al.* It is also important that the appearance (next week) of the article from the Brigham Young group should not be taken to imply that all those who have seen it are persuaded to its chief conclusion. Rather, on the advice of the referees, the authors have produced an article which answers questions that would otherwise spring to readers' minds. That, all honour to them, is what referees are for. □

# Scientific look at cold fusion inconclusive

- International meetings produce interest
- Laboratories struggle to replicate findings

## Dallas

THE tantalizing prospect of 'cold fusion' continued to galvanize interest around the world last week with two international conferences making the first public professional assessment of the results while daily newspaper stories continued to describe rumours, reports and retractions of cold-fusion experiments.

In Dallas, a 10,000 seat basketball arena was filled to near capacity on Wednesday as chemists attending the national meeting of the American Chemical Society (ACS) gathered to hear the latest results of experiments by Stanley Pons of the University of Utah. At the same time in Erice, Italy, a smaller gathering made up predominantly of physicists witnessed the first confrontation between Martin Fleischmann, Pons's colleague, and Brigham Young University's Steven Jones, who has also performed cold-fusion experiments. The two universities have been competing for primacy in the cold-fusion arena.

Since the University of Utah announced its results at a press conference on 23 March, laboratories all over the world have been trying to replicate the relatively simple experiments. Pons and Fleischmann use palladium and platinum electrodes in a lithium electrolyte solution of deuterated water. When a current is applied to the cell, deuterium is taken up by the palladium cathode, and, according to the University of Utah team, can fuse, releasing energy. So far, no one has reported unequivocal confirmation of the Fleischmann/Pons results.

But that did not dissuade some 7,000 chemists, as well as a gaggle of journalists and members of the public, from sitting in rapt attention as Pons described his experiments. ACS decided at the last minute to hold a special forum on cold fusion, but society officials were quick to point out that the decision did not constitute any validation of the experimental results.

At Dallas, chemists welcomed the prospect that cold fusion might represent a victory for chemistry over physics. Opening the special session, ACS presi-

dent Clayton Callis said the goal of fusion as an energy source has remained elusive, and that physicists' efforts at hot fusion using tokamaks and lasers were "apparently too expensive and too ambitious to lead to practical power". To applause from the crowd, he added, "Now it appears that chemists have come to the rescue".

In his speech, Pons joked about the high cost of physicists' attempts at fusion by calling his own apparatus the "U-1 Utah tokamak". A picture showed a glass electrochemical cell inside a cooling bath made from an ordinary rubber kitchen bucket.

But Harold Furth, director of the Princeton University Plasma Physics Laboratory, raised serious questions about the conclusion that Pons was seeing fusion. Furth said that the experimenters would have to show that the same reaction did not occur with light water rather than deuterated water before nuclear physicists would try to explain why the deuterium was fusing inside the palladium lattice.

Scepticism for fusion as the explanation for the heat seen by the University of Utah team was also expressed at the Erice meeting (see page 616).

At a meeting between Fleischmann, Jones and Pons as well as the presidents of BYU and the University of Utah on 6 March, both teams agreed to submit on 24 March papers describing their results to *Nature*. But when the University of Utah team held a press conference on 23 March, the BYU team submitted their work to *Nature* the same day.

Now both universities are working to prove that their people were the first to show that relatively high rates of fusion could occur in an electrochemical cell. The Pons/Fleischmann results have been far more dramatic than those reported by

Jones, with claims that the electrochemical cell produces a large excess of heat. Jones's paper will appear in the next issue of *Nature*.

At the Italian meeting, Jones and Fleischmann were cordial towards one another, and press photographers eagerly snapped pictures of the two smiling for the cameras.

Daily newspapers and television programmes continue to be a favoured forum for reporting new scientific results. On 10 March, Texas A & M researchers held a press conference to say that they were seeing between 60 and 80 per cent excess heat production using an electrochemical cell similar to the one at the University of Utah. On the same day, Georgia Institute of Technology scientists held a press conference to say that they had produced neutrons and tritium from a similar experimental apparatus, but by the end of the week they had to retract that claim, citing problems with the instruments used to detect the neutrons.

On Wednesday, 12 March, reports surfaced in the Western media that the University of Moscow had successfully replicated the experiment, but no details have been forthcoming (see page 607). The same day, rumours circulated that Massachusetts Institute of Technology (MIT) professor Peter Hagelstein had submitted four theoretical papers explaining how fusion could occur without generating a lethal amount of radioactivity.

MIT has subsequently released a summary of the papers and filed a patent application dealing with their possible technical application. MIT also announced that another MIT professor, Keith Jones, had a theory that explained the Utah results without resorting to a new theory of nuclear fusion.

Although there is uncertainty about the University of Utah results even among those inclined to believe them, financial support for the work has grown. The Office of Naval Research (ONR) has awarded the University of Utah team an additional grant of some \$400,000 over the next 32 months for work on cold fusion, nearly doubling ONR support for Pons' laboratory. □

Dick Thompson

IMAGE  
UNAVAILABLE  
FOR COPYRIGHT  
REASONS

## Erratum

A news item in the 13 April issue of *Nature* (338, 529; 1989) incorrectly referred to Brigham Young University researcher Steven Jones as Robert Jones. The same article also cited the publication date for an article in the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* by Stanley Pons and Martin Fleischmann as 10 March. The correct date is 10 April. □

After press conference in Dallas, Stanley Pons (wearing glasses) is pursued by reporters, protected by police.



# Japan no help to rain forests

## Tokyo

JAPAN is severely criticized for its role in the destruction of the world's tropical rain forests in an analysis of its tropical timber trade released last week by the Worldwide Fund for Nature (WWF, still known in the United States as the World Wildlife Fund).

The report, compiled for WWF by a French economist and the head of the Japan Tropical Forest Action Network (JATAN), is the first comprehensive analysis of Japan's tropical timber trade, which accounts for nearly a third of the world market. It claims that over the past 30 years, a combination of Japan's trade and foreign aid policy, a booming economy and wasteful consumption of wood has led to the systematic destruction of rain forests in much of South-East Asia.

Japan's overseas development assistance (ODA), now the largest in the world at \$11,000 million in 1989, comes in for particularly severe criticism in the report. Funds have been used to finance the construction of dams and roads (often designed and built by Japanese companies) which have destroyed rain forests and damaged the health and economic welfare of local populations in several South-East Asian countries.

The report criticizes the management of ODA, claiming there is no central organization coordinating Japan's aid policy and that responsibility is dispersed among several ministries and agencies. The Ministry of Foreign Affairs is supposed to play a coordinating role but other ministries, in particular the Ministry of International Trade and Industry and the Ministry of Agriculture, Forestry and Fisheries, have a strong voice in many ODA projects. Despite the large number of agencies

involved, there is a lack of trained ODA staff in Japan compared with other developed nations.

Japan's ODA projects also lack adequate environmental impact assessments, according to the report. Assessments for ODA projects were introduced in 1980 but are voluntary and confidential and lack the influence to do more than modify a project.

The report calls on the Japanese government to establish binding environmental impact assessments that will be open to public discussion. It recommends a committee of representatives of related ministries, the timber industry and non-governmental organizations (NGOs) be set up to formulate a plan of action for conservation of tropical rain forests, and a code of conduct be written that would restrict trade to timber from sustainable resources. The report urges Japan's industry to follow recent proposals from the European timber industry and contribute to a fund to promote sustainable forestry through the International Timber Trade Organization. Finally, the report calls for a nationwide campaign to reduce Japan's wasteful and unnecessary consumption of tropical timber, particularly in the paper and construction industry.

The WWF report comes at a time when there is growing concern worldwide about the effects of rain-forest destruction on global climate. But, as the report points out, there are very few people within Japanese government ministries and agencies who have expertise in assessing the environmental impact of the timber trade and ODA on rain forests. Even Japan's NGOs, including WWF Japan, lack such experts.

**David Swinbanks**

## UNIVERSITY DISPUTE

# Research councils to award studentships

## London

THERE is no end in sight to the 14-week-old dispute over university lecturers' pay after members of the lecturers' union, the Association of University Teachers, voted by a narrow margin to reject the latest offer of the Committee of Vice-Chancellors and Principals. In a ballot held last week there was a 70 per cent turnout; 47 per cent of members voted in favour of accepting the offer of a 6.5 per cent pay increase, and 53 per cent voted against.

Disruption of examinations is likely to be minimized, however, now that the union is considering reducing the action from a boycott of all work related to examinations to a boycott only of marking examination papers. The union's council will decide on 22 April whether to accept this recommendation from its executive.

A boycott of marking will cause a delay in the announcement of degree results. This poses problems for the research councils in their allocation of postgraduate research studentships, as these depend on students receiving a first or upper-second class degree. If results are not available before the end of July, four of the councils (Science and Engineering, Natural Environment, Medical Research, and Agricultural and Food) have agreed that studentships will be awarded on provision of a written guarantee from heads of department or from vice-chancellors, saying that on the evidence available from continuous assessment and referees' reports the student is likely to gain the required standard of degree. The Economic and Social Research Council may adopt a different procedure.

**Christine McGourty**

# Z<sup>0</sup> factory makes ready

## Berkeley

THE Stanford Linear Collider (SLC) passed a major milestone on 12 April, with the production of its first Z<sup>0</sup> particle. Repairs carried out over the autumn and winter have boosted the instrument's reliability tenfold and Stanford Linear Accelerator Center (SLAC) officials expect to produce several thousand Z<sup>0</sup> particles by the end of the summer, enough to determine the mass of the particle and to begin to analyse its lifetime and decay modes.

SLC uses a pair of curved arcs to guide electron and positron bunches from a linear accelerator onto a collision course. The design, conceived by SLAC director Burton Richter, provides an inexpensive alternative to the circular Large Electron-Positron Collider (LEP) due to come on line this summer at CERN in Geneva.

Although SLC began with a two-year lead over LEP in the race to characterize the Z<sup>0</sup> particle, most of that time has been lost overcoming technical difficulties in commissioning the new machine. The beams first met last spring, but high background in the detection chamber and low reliability of operation forced the shutdown of the machine for repairs last September (see *Nature* 335, 285; 1988).

During last autumn and winter, new magnets called muon spoilers were added to SLC, to deflect background muons away from the detector. A slight misalignment in the damping ring where positrons are stored was also discovered and repaired.

Last week, SLC began running at an efficiency that should produce one Z<sup>0</sup> particle per day, according to SLAC spokesman Michael Riordan. But Richter warned that "many months of hard work lie ahead" before it will be operating at design specifications, which should produce 3,000 Z<sup>0</sup> particles per day.

SLC's design specifications call for 60,000 million electrons and positrons per bunch, with a bunch radius of 1.6 µm, and a pulse rate of 120 per second. The machine is now running at 15,000 million electrons or positrons per bunch, with a bunch radius of 4 µm and a pulse rate of 30 per second. Riordan said fine tuning over the next few months is expected to bring down bunch radius and background, increasing the number of Z<sup>0</sup>s produced. But several additions will be needed before the instrument can operate at full power. A new cooling tower, new positron source and additional power for the damping rings will be completed and installed in the autumn and superconducting magnets for narrowing the beams added later on. The additions scheduled for this year are all within the SLC's \$100-million budget for 1989, said Riordan, although support for 1990 has to be found.

**Marcia Barinaga**

## CANCER

# Inquiry into Pasteur deaths

## Paris

THE death from cancer earlier this month of 38-year-old Willem Roskam, a former researcher at the Pasteur Institute in Paris, has added poignancy to an inquiry into health hazards at the institute. Roskam was one of seven Pasteur researchers to have developed rare forms of cancer (in his case, lymphoblastic lymphosarcoma). All had worked on the same floor, at about the same time and all had been involved with research using recombinant DNA. Three of the other researchers have also died as a result of their illness. But an inquiry being carried out under the direction of Professor Jean Bernard is not expected to report until the end of the year.

By 1986, five cases of cancer had developed and by 1987 the number had risen to seven. "We began to ask ourselves some questions", says Maxime Schwartz, director of the Pasteur Institute. In 1987, the Pasteur decided to undertake an inquiry to determine epidemiological factors that could distinguish the cancer victims from a large population of present and former workers at the institute. A questionnaire was sent out to more than 4,000 researchers and other staff who had worked at the institute for at least six months between 1971 and the end of 1986 to determine which chemicals they used, which microorganisms they were in contact with and in which parts of the institute they worked.

"People are surprised at the time the inquiry has taken", says Schwartz, "but we needed to find the names and

addresses of all these people, contact them and then wait for the replies". Now, says Schwartz, the National Institute for Health and Medical Research (INSERM) has enough replies for a representative sample, but the analysis will not be complete before the end of the year.

So far, the only link between the seven victims is that they worked in neighbouring laboratories. But, says Schwartz, "the Pasteur Institute has always taken enormous care with safety in the handling of dangerous substances. In 1986 we reinforced these safety measures by creating new norms for the occupancy of laboratories. Before I became director, the safety officer and I visited each laboratory and determined the maximum number of people who could work in each room. Now we accept or refuse new visitors according to these norms."

Roskam stayed at the Pasteur Institute for only two years, from 1977 to 1979, where he worked on the production of growth hormone using genetic-engineering techniques. He later became research director at Sanofi-Elf-Biorecherche. If the inquiry indicates a link between the cancers and the victims' occupation, says Schwartz, the problem may well turn out to apply elsewhere.

As a result, the Pasteur Institute is taking part in a wider epidemiological study being carried out by a Lyons-based cancer research institute, involving laboratories in 13 countries, to see whether the incidence of cancers is higher in the laboratories than in the population at large.

**Peter Coles**

## COLD FUSION

# Mixed success in East

## London

SEVERAL teams in the socialist bloc have reported replicating the 'cold-fusion' experiments of Pons and Fleischmann (see page 605). Not for the first time, the Hungarians were the first off the mark.

On 30 March, only six days after the original announcement, Academician Gyula Csikai and Dr Tibor Sztaricsai of the Kossuth Lajos University of Debrecen managed to replicate the experiment, using platinum and palladium electrodes.

The experiment lasted for such a short time that they could not measure the energy outflow, but their neutron count convinced them fusion had taken place.

In Poland, two teams have reported positive results, one at the Technical University of Gliwice, and one at the University of Wroclaw, while a third team, at the Institute of Plasma Physics and Laser Microfusion in Warsaw, is also working on the problem — so far, without success.

The Poles seem, on the whole, less confident than the Hungarians — Professor Lech Pajdowski, head of the Wroclaw team, said that his experiment, which lasted for 1,000 seconds, indicated a definite reaction, but that it was impossible to state unequivocally that this was fusion. He also said that, according to one of his students, a similar result had been reported by German researchers in the 1920s.

In the Soviet Union, positive results have been reported from Moscow University and from Kharkov, which claims that fusion has been achieved at temperatures between  $-130^{\circ}$  and  $-150^{\circ}$  Centigrade. That seems, to say the least, out of step with other claims.

**Vera Rich**

## SPACE APPLICATIONS

# Private laboratory plans premature

## Washington

PLANS for a privately developed space laboratory for microgravity research and manufacturing which were laid under the Reagan administration should be abandoned, according to a report\* released last week from the National Research Council. The report says that the space shuttle can accommodate the "vast majority" of planned experiments and that there is "no evidence to suggest that microgravity research would lead to significant space-based manufacturing in the next five to 10 years".

The report deliberately avoids joining the debate over the role of "commercial development" in space research. But its conclusions on microgravity research, the most promising area for commercial exploitation, are bound to weaken the Reagan-era view that space is "just another place to do business".

Two years ago, a Houston-based com-

pany, Space Industries Inc., put forward the plan for an orbiting 12-metre-long microgravity capsule that could be launched by the space shuttle in the early 1990s. Automated research and manufacturing facilities would have been inspected by astronauts several times a year.

Although the laboratory was intended to provide commercial opportunities in the period before NASA's manned space station Freedom is completed at the end of the century, private industry remained unenthusiastic. NASA opposed the laboratory on the grounds that it would compete with the space station for funds.

But strong support for an orbiting spacecraft "financed, owned and operated by the private sector, rather than by the NASA" came from the Reagan administration in 1988 and plans were made for the government to back the project by leasing most of the laboratory.

The National Research Council, the

investigatory arm of the National Academies of Science and Engineering, was asked to examine the prospects for the laboratory in late 1988. Its report accepts the value of a low-gravity environment for research but sees no need for a commercial space laboratory. Already existing facilities can provide a range of gravity-free conditions, from seconds at a drop tower, through minutes on an inexpensive sounding rocket, to a full week on the space shuttle. From around 1992, extended shuttle missions will provide for experiments of 16 to 28 days' duration, adequate for "virtually all" planned microgravity experiments.

According to the report, since basic problems still need to be solved, "human-tended" rather than automated laboratories are needed now. But a commercial space manufacturing facility will make sense further in the future, when telerobotic systems and computer-assisted decision are more advanced.

**Alun Anderson**

\*Report of the Committee on a Commercially Developed Space Facility, National Academy Press, 1989.



## British-German collaboration agreed

Munich

A NEW programme to promote research collaboration between West German and British institutes of higher education has been launched by the British Council and the German Academic Exchange Service (Deutscher Akademischer Austausch Dienst or DAAD). The programme will have an annual budget of £350,000.

The programme, entitled the Academic Research Collaboration, will support 80 multi-year projects in science, engineering and business studies undertaken by researchers in British universities and polytechnics and in West German universities and *Fachhochschulen*. The intention is to provide several members of a research group with the chance to work in the other country.

The Anglo-German Foundation for the study of Industrial Society, based in London, will provide an additional £30,000 in 1989 for collaborative research in the social sciences. The British Council office in Cologne will handle applications from British researchers; the DAAD headquarters in Bonn will handle applications from West Germany.

Steven Dickman

## SCIENCE JOURNALS

### What's in a name?

Paris

THE decision of the Pasteur Institute to give its journal of microbiology, virology and immunology an English title is continuing to create protest in the correspondence columns of the national newspaper *Le Monde*, to the exasperation of the institute's director, Maxime Schwartz (see *Nature* 338, 448; 6 April 1989). The venerable Académie Française condemned the decision outright, the distinguished scientist Axel Kahn called the move "suicidal", and even President François Mitterrand asked for an explanation.

But most vociferous have been Canadian scientists from Quebec, proud of their success in keeping French alive against all odds. "If the French abandon their mother-tongue", said an irate Canadian at a press conference last week, "what hope is there for us or for African countries?" Obviously tired of repeating himself, Schwartz politely pointed out that out of the 2,500 subscriptions, only about 75 were from Canada and fewer than 10 from Quebec.

Research Minister Hubert Curien told *Nature* that the affair had grown out of proportion, asking that the French set an example to other nations by speaking French "out of love for the language, not out of laziness". Meanwhile, the Pasteur Institute stands firm on its decision but plans to publish a new review in French with original scientific notes.

Peter Coles

## Soviets readmitted to WPA

London

THE Soviet psychiatric establishment has been provisionally readmitted to membership of the World Psychiatric Association (WPA) pending the WPA's six-yearly congress in Athens next October. The Soviet All-Union Society of Psychiatrists and Neuropathologists resigned from the WPA in 1983, anticipating motions for its expulsion on the agenda of the WPA Congress in Vienna that year. At the 1983 congress, it was understood that the Soviet psychiatrists would be welcome to apply for readmission if and when they gave sufficient proof that the systematic use of psychiatric methods for the repression of sane political dissidents and religious believers had ceased in the Soviet Union. The Soviet psychiatrists now maintain that this condition has been met. A number of Western psychiatric associations, including the Royal College of Psychiatrists in Britain and the West German Deutsche Gesellschaft für Psychiatric und Nervenheilkunde, maintain that their readmission would be dangerous and premature.

The provisional reinstatement of the Soviet organization was sponsored by the president of the WPA, Dr Costas Stefanis, and its general secretary, Dr Fini Schulsinger, who invited two Soviet representatives to attend a recent meeting of the European regional executive of the WPA in Granada. After speeches from these visitors, one of whom, Dr Nikolai Zharihov, maintained that the Soviets had now mended their ways, while the other, Dr Protr Morozov, claimed that there had never been any misuse of psychiatry anyway, Stefanis and Schulsinger pushed through the motion of reinstatement, over the protests of those executive members who felt it would be more appropriate to wait for the report of the recent fact-finding and 'verification' mission of US psychiatrists to the Soviet Union.

The motion of reinstatement contravened a number of procedural rules of the WPA. In particular, the application for readmission (which according to rule had to be filed a year before the next congress) was filed by the All-Union Soviet of Psychiatrists and Neuropathologists. Shortly afterwards, however, the neuropsychologists seceded from this society and set up their own organization, while their place was taken by the narcologists, so that the organization which was provisionally admitted to the WPA in Granada — the All-Union Society of Psychiatrists and Narcologists — was not, formally speaking, the same organization that had applied last October.

This technicality acquired more than a formal significance, as Stefanis and Schulsinger told the executive meeting that they

had rejected out of hand the application for membership of the new unofficial Association of Independent Psychiatrists established in Moscow last month, on the grounds that its application had come too late for consideration at the next WPA Congress.

Technicalities apart, the main issue is whether the official Soviet psychiatric profession has mended its ways. In spite of high-sounding legal commitments, the handing over of the administration of the former special psychiatric hospitals from the jurisdiction of the police to the Ministry of Health and some press articles deploring the occasional "hyper-diagnosis" of dissidents during the Brezhnev era, the Netherlands-based International Association on the Political Use of Psychiatry has collated an impressive body of evidence suggesting that patients are still being held in Soviet psychiatric hospitals on political grounds.

The promised report of the US fact-finding mission, which it is now said will not be ready until June, seems unlikely to clarify the issue. There seems to have been an agreement between the US visitors and their Soviet hosts that neither side will make statements to the media based on the results of this mission. The Soviet side has in fact already breached this agreement, by claiming that the US experts found that all was well with the Soviet psychiatric practice — a statement which many of the visitors maintain did not reflect their views.

Vera Rich

## NUCLEAR DECOMMISSIONING

### MPs criticize delay

London

THE decommissioning policy of Britain's nuclear industry was severely criticized by the House of Commons Energy Committee last week. After examining the annual report and accounts of British Nuclear Fuels Limited, the committee says the decision to postpone decommissioning of nuclear plans for 50 to 100 years after closure may have been adopted in order to bolster the company's short-term profits, and it is sceptical of the company's denials. The committee suggests that the Nuclear Installations Inspectorate be asked to assess whether there are any safety, environmental or technical reasons for postponing decommissioning for longer than 20 or 30 years. The committee directs the same criticism at the electricity boards, and says the government should clarify how the costs of decommissioning nuclear power stations would be met if these boards, soon to be privatized, went into liquidation.

Christine McGourty

## SUPERCOMPUTERS

# Japan and US both claim lead

## Tokyo

NEC Corporation of Japan last week launched what it claimed to be the world's fastest supercomputer, raising fears in the United States that Japan has taken the lead in this key technology. But Cray Research Incorporated, the world's leading supercomputer manufacturer, is unperturbed. Officials of the US company's Japanese subsidiary say that Cray's fastest computer can already match the NEC supercomputer and that a new model will leave its Japanese competitor behind.

On the face of it, the top model in NEC's new SX-3 series of supercomputers is faster than anything Cray or anyone else has to offer. NEC's model SX-3/44, which has four central processing units, can, in theory, churn out 22,000 million floating-point operations per second (flops), a speed that is more than five times faster than Cray's fastest computer, the Y-MP 8/8 (see below). But in practical use, when computers operate far below peak speed, Cray may have the advantage.

Takehiko Kato, an engineer at Cray Research Japan Ltd, has simulated the performance of the two computers for a range of operating conditions. He claims that NEC's computer is comparatively slow at scalar processing (calculations on individual numbers) and can gain an advantage over Cray's computer only when performing calculations where vector processing (simultaneous calculations on lists of numbers) can be used more than 85 per cent of the time.

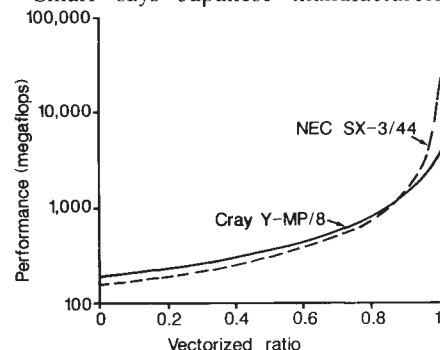
That means a Cray will be faster on almost all practical applications, including drug and chemical design, structural engineering and aerodynamics. Only in special fluid-dynamic calculations where vector processing exceeds 85 per cent will the high speed of NEC's computer be an advantage, Kato says. His simulation relies, however, on an estimate of 160 million flops for the NEC computer's scalar processing speed. NEC refuses to reveal a precise figure but claims Kato's estimate is "misleading".

Speed is only one of many factors considered by purchasers of supercomputers, according to Larry Smarr, director of the National Center for Supercomputing Applications in the University of Illinois at Urbana-Champaign. And speed comes comparatively low on the list of users' priorities if switching to a new system would require rewriting of software and computer codes (as a switch from Cray to NEC would require). A more important consideration, Smarr says, is the floating-point standard which the two competitors have adopted for their supercomputers.

Cray uses the IEEE standard that has been generally adopted by US scientists

and engineers. But Japanese manufacturers, including NEC, are using the IBM standard for their supercomputers. Smarr says that as long as the Japanese keep to that standard, he would not buy one of their computers even if it were infinitely faster than a Cray because of software problems and incompatibility with computers already linked to his centre's supercomputer network.

Smarr says Japanese manufacturers



focus on IBM as their main competitor but this attitude is "schizophrenic" when at the same time they are trying to conquer the world of supercomputing, where IBM is a comparative outsider.

Nevertheless, Smarr says NEC's new computer has put Cray on a "tightrope" because the stockmarket may be "skittish" about any delay in the introduction of Cray's next model, the Cray-3. Cray officials, on the other hand, say the Cray-3 will give the company a decisive lead over NEC.

The Cray-3, which is expected to be on the market around the same time as NEC's new computer (shipments of which will begin in June next year), will be the first supercomputer to use logic chips made of the ultra-fast gallium arsenide, rather than silicon. It will be able to execute calculations much faster than NEC's new model because of its higher scalar processing speed (close to 1,000 million flops), Kato says, despite a lower top speed for vector processing (16,000 million flops).

But pricing strategies and technical back-up will be important in determining market share, particularly in Japan. Japan's huge electronics manufacturers have managed to seize the main share of the domestic market by aggressively slashing prices, something which a comparatively small company like Cray cannot afford to do. Nevertheless, despite a widely held perception that US supercomputers have fared badly in Japan, Cray has captured 40-50 per cent of the market in the private sector, Kato says. And some Japanese car manufacturers have recently switched to Cray supercomputers for use in car design.

Cray has not done so well in the public

sector, selling only two supercomputers to government research organizations since bidding was made more open to foreign companies in 1987. But most analysts expect the bulk of supercomputer sales in the future to be in the private sector, particularly in Japan and Europe where there are few large national research centres comparable to those of the United States. Smarr says that as supercomputers become cheaper they will begin to "percolate down into the Fortune 500 companies" from the giant corporations that use them now.

Cray and NEC agree with this assessment. NEC hopes to sell about 120 supercomputers in Japan and overseas over the next four years with 60 per cent going to the private sector. And Cray expects the Japanese market to more than double to 250 computers during the same period, again with the bulk of the sales in the private sector.

David Swinbanks

## EUROPEAN SPACE PROGRAMME

## Cornerstone laid in West Germany

## Munich

WEST Germany has begun the expansion of the ground control centre at Oberpfaffenhofen, near Munich, for future space station and spacelab missions. The foundation stone for the new centre was laid on 4 April. Construction will proceed in two phases.

The West German Research Ministry will invest DM85 million by 1991 to prepare for the next West German spacelab mission. A new computer centre and robotics centre will also be created. The European Space Agency will then invest an estimated DM100 million to build a control centre for the European space station Columbus.

Steven Dickman

## AUSTRALIA

## Science fiction for science students

## Sydney

SCIENCE students at Murdoch University in Western Australia are to be given a foundation course in science fiction in an attempt to wean them from television and videos. The only books they ever read are textbooks, according to their course coordinators, creating a "literacy problem".

The course on 'Life and the Universe' will include *Dune* by Frank Herbert and *Ringworld Engineers* by Larry Niven in its reading material. One of the course organizers, David Macey, says that students are "reluctant to acknowledge they have a problem with literacy. A short science fiction novel can move them sideways into general reading and general biological concepts."

Tania Ewing



# Big drug company created

## Washington

THE UK pharmaceutical concern Beecham Group plc and the US drugs and scientific instruments company SmithKline Beckman last week announced plans to merge in a move that could make them the world's second largest pharmaceutical company in terms of sales.

But despite rumours that the anticipated merger will lead to layoffs of scientific personnel as the new company consolidates its research programmes, a SmithKline Beckman spokesperson says there will be no cutbacks in research.

In the intensely competitive \$127,000 million worldwide market for pharmaceuticals, SmithKline Beckman and Beecham Group plc have both had stock problems that have made them potential takeover targets in the past year. After hitting its peak as the world's best-selling drug in 1986, SmithKline Beckman's ulcer drug Tagamet experienced an unexpected slump in sales beginning in 1987. Beecham Group plc is just beginning to see the fruits of its economic recovery in both prescription drug sales and over-the-counter preparations and toiletries, after encountering a series of difficulties in the early 1980s.

After the merger, the new company, SmithKline Beecham, will be virtually untouchable by corporate raiders. Even though neither company has put a price on the deal, analysts estimate the combined company will be worth about \$16,000 million and will have annual sales of roughly \$5,000 million.

Separately, neither SmithKline Beckman nor Beecham Group ranks within the top ten drugs companies, but together, SmithKline Beecham may have sales second only to Merck. With a combined sales force of more than 6,000, SmithKline Beecham hopes to capitalize on the creation of a single European market in 1992, and to compete more effectively in Asia.

The merger is expected to strengthen the research and development programmes of both companies. A planned research and development budget of \$500 million will boost SmithKline Beecham into the "first tier" of pharmaceutical companies in terms of research and development expenditures, says Niel Sweig of Prudential-Bache Securities. But he says it will still be way behind Merck, which is due to spend \$755 million on research this year.

The combined company will employ 4,800 researchers, spread out over centres in Pennsylvania and Great Britain, including a \$200-million research and development complex in Pennsylvania opened by SmithKline Beckman in 1986. No research jobs are expected to be

eliminated by the merger; the 10,000 layoffs expected in the combined company's 70,000-person workforce will probably come from company administration and pharmaceutical production activities.

As part of the merger, SmithKline Beckman will 'spin off' its Beckman Instruments subsidiary, which means that Beckman Instruments will become a separate company owned entirely by public stockholders, not a parent company. The company's 400 researchers, who work in designing and testing Beckman's centrifuges, chromatography instruments and clinical diagnostic equipment, are expected to remain unaffected. In an internal announcement to employees, Beckman Instruments

## SAFETY REGULATIONS

### Fears on costs of new requirements

#### London

RESEARCHERS in Britain's universities and research institutions will have to give higher priority to safety to meet the requirements of new legislation which comes into force in October. Before beginning research, they will need to carry out a formal assessment of the risks involved in using any substances that may be hazardous, a term so all-embracing that it includes everything from arsenic to sugar.

The new regulations, called COSHH (Control of Substances Hazardous to Health), bring research activities under much more strict statutory control. Apart from specific controls relating to, for example, the use of carcinogenic substances or ionizing radiation, the only legal obligation on employers has been to ensure the safety of employees as far as reasonably practical. Now, they must ensure that a risk assessment is carried out and exposure to hazardous substances prevented or controlled.

University safety officers, glad to have the force of law more firmly behind them, have welcomed the COSHH regulations and say that present safety procedures need not be altered significantly, just carried out more systematically.

The safety officer for King's College, London, Roger Slade, says most universities already adhere to the required safety standards. But researchers are less than enthusiastic about carrying out the risk assessments, which they fear will be time-consuming, and universities fear the costs of taking on extra administrative staff as well as of safety hardware such as fume cupboards.

Despite a huge information campaign by the Health and Safety Executive, there is still confusion among researchers about how detailed the risk assessment will have

to be. A working party of the Institute of University Safety Officers has now formulated guidelines on assessment which are being sent to universities this week. In supervised practical teaching of undergraduate and postgraduate work, no assessment specifically to meet the COSHH requirements will be necessary because safety should already be included in existing written protocols for this work, says the institute. And when standard research techniques are being used, no assessment for COSHH will be necessary.

Only when new substances and non-standard techniques are used, or when an experiment involves a "significant" risk, will a full written assessment be necessary. (Another report is being produced on how to identify research that poses a "significant" risk.)

Christine McGourty

Carol Ezzell

## SPACE

### NASA chief named

#### Washington

REAR Admiral Richard Truly has been named to succeed James Fletcher as administrator of the National Aeronautics and Space Administration (NASA). A former astronaut, Truly is now head of the office of space flight at NASA. J. R. Thompson, director of the Marshall Space Flight Center in Huntsville, Alabama, is to be nominated as deputy administrator.

Before becoming NASA administrator, Truly must clear two hurdles. First, his nomination must be confirmed by the US Senate, and second, Congress must pass a waiver permitting a former military man to head the civilian space agency. Truly is now on active status in the Navy, but will resign his commission. Neither hurdle is expected to be difficult to clear.

Joseph Palca

## NUCLEAR POWER

# Wackersdorf to be buried

## Munich

CONSTRUCTION of West Germany's controversial nuclear fuel reprocessing plant at Wackersdorf may have been rendered superfluous by a non-binding agreement, made public last week, that would allow West German fuel to be reprocessed at La Hague in Normandy. Following a meeting between West German Chancellor Helmut Kohl and French President François Mitterrand planned for this week, a sudden decision could be made to abandon the plant.

The agreement to reprocess West German nuclear fuel at La Hague was announced between the West German energy company VEBA AG and the French state-owned nuclear fuels company Cogema. It was the first time that Cogema had offered a West German company the chance to share ownership in its expanded reprocessing plant at La Hague. VEBA, which owns Preussen Elektra, the second-largest electric utility in West Germany, would take more than 49 per cent ownership of the third 'plutonium factory' (Usine Plutonium 3 or UP 3) at the Hague beginning in 1999. UP 3 is expected to be completed by 1991.

The agreement would provide for reprocessing of at least 400 tonnes of spent fuel a year (the amount used by Preussen Elektra plants). In addition, Cogema would also reprocess fuel used by all other plants in West Germany at "fair and reasonable prices", said VEBA spokesman Henrich Wilckens. The cost to VEBA is rumoured to be in the range of DM 2,500 million, roughly a third of what is needed to complete Wackersdorf.

West German utilities already have contracts with Cogema to reprocess fuel elements but they are expected to end in the mid-1990s, when Wackersdorf is planned to begin operation.

The decision to abandon Wackersdorf would be an about-face for Kohl's centre-right coalition government. Kohl has until now held fast to the 'national concept' of

breeding and reprocessing nuclear fuel, as well as storing nuclear waste, in West Germany.



Building the Wackersdorf plant has brought more than 1,600 jobs to the economically depressed region of Oberpfalz in northern Bavaria. Kohl expressed strong support for Wackersdorf as recently as last November.

The Kohl government, under fire from the Bavarian government, refused last week to be pinned down, saying only that a "new situation" has arisen. But political analysts see a golden opportunity for Kohl to rid himself of one of his heaviest political burdens and counter his waning popularity by burying Wackersdorf.

Bavaria has demanded compensation should the project be shelved. More than DM2,000 million has already been invested. So far, the Bavarians have rejected the suggestion that the plant be used as an intermediate or final storage site for nuclear waste.

Wackersdorf has been a rallying point for anti-nuclear demonstrators. More than 3,000 people have been tried in local courts in the four years since the decision to begin construction. German courts are still burdened with cases protesting at the construction on constitutional and other grounds (see *Nature* 333, 8; 1988).

By abandoning Wackersdorf, VEBA would save in court costs and in direct investment, which would allow electricity prices to be kept down while creating a better image for VEBA. And the international tie-up would also help to prepare for the creation of the European Single Market in 1993.

Nuclear power plant builders are already merging their operations. The West German and French nuclear power plant producers Kraftwerk Union (KWU) and Framatome announced last week the formation of a common subsidiary to be based in Paris. The new company 'Nuclear Power International' will sell reactors to third countries.

Steven Dickman

## OIL SPILL

# Environmental effects still unclear

## Berkeley

IN the third week following the wreck of the *Exxon Valdez* in Alaska's Prince William Sound, north-east winds carried much of the oil slick into the Gulf of Alaska where rough seas helped to break it up.

Jacqueline Michel, a biologist with the National Oceanic and Atmospheric Administration, says that early fears that toxic hydrocarbons would dissolve in the water were not realized.

Analysis of water samples taken from the sound a week after the spill detected no aromatic hydrocarbons or volatile organic compounds. The first sample of plankton taken from one affected part of the sound seemed normal as well, says Michel.

Herring have begun spawning in the sound, but there is no indication of whether the fish or their eggs have been harmed by the spill. The 1,300 dead birds and 200 dead sea otters that have been recovered are only the tip of the iceberg, says Michel, and there is still concern that the oil will seriously affect the migratory seabirds that are just beginning to nest on islands in the path of the slick.

Marcia Barinaga

## BLOOD PRODUCTS

# All suspect in India

## New Delhi

THE Indian government has ordered the withdrawal and destruction of all locally made blood products and banned their production until further notice. The director general of the health services has also appealed to all those who have used these products to report to the nearest government laboratory for testing for antibodies to the human immunodeficiency virus (HIV).

The government action followed the recommendations of a committee set up in February following the chance discovery of HIV antibodies in samples of anti-rh immunoglobulin marketed by a Bombay company. The committee found that blood products manufactured by two other companies were also contaminated. Further investigation showed that all the nine companies in the country had been flouting rules regarding screening of donor blood for HIV. Their production technology was found incapable of ensuring inactivation of the virus.

The list of banned products includes 16 items made from blood and human placenta. The companies involved have been told that their licences will be suspended until they modify their production process. Until then, the entire demand for blood products will have to be met through imports. Imported products must be accompanied by certification showing them to be free of HIV and are subject to screening in India.

K.S. Jayaraman

## SOVIET UNION

# Forest ministry felled

## London

THE government of the Uzbekistan Republic chose 1 April to announce the abolition of its Ministry of the Forestry Industry. This decision, the official communique stressed, was not an April Fool's Day joke, although the fact that such a ministry had ever been set up may have been. For Uzbekistan is 80 per cent desert and barren mountains, and the only woodland is located in a few national parks and reserves administered by the State Commission for Nature Conservation.

Vera Rich



# Heroes and villains

SIR—Benno Müller-Hill is known not only as a specialist in molecular biology but also as someone involved in establishing the personal responsibility of scientists for inhuman experiments under the Nazis. In his review of Daniil Granin's book (*Nature* 336, 721; 1988) about Nicolai Timoféeff-Ressovsky under the heading "Heroes and Villians", he confirms that Timoféeff-Ressovsky did not participate in such experiments. At the same time, Müller-Hill implies that Timoféeff-Ressovsky, contrary to Granin's opinion, was not a heroic person, because, as head of the genetics department at Kaiser Wilhelm Institute, he had business contacts with proponents of 'racial hygiene'. (Müller-Hill later testifies that Timoféeff-Ressovsky did not himself publish a single anthropological paper.)

An objective look at the past together with a trustworthy evaluation of the personal conduct of those living under totalitarian regimes is very important. Timoféeff-Ressovsky is unique — he had to live and work both in Hitler's Germany and Stalin's Soviet Union.

In our opinion, in order properly to understand and evaluate the life and deeds of Timoféeff-Ressovsky, it is not sufficient to see them through the eyes of somebody living much later in a comfortable democratic society. For example, Pyotr Kapitsa, when rescuing the persecuted Lev Landau, wrote a letter to Stalin. Its content and especially its form seem unattractive by the moral standards accepted in Western democratic societies: in this letter, the prominent physicist humbly asks Stalin to release his guiltless colleague from prison and promises that Landau will not in future act against the state. But taking into account the situation in the Soviet Union at that time, this was an act of heroism by Kapitsa because it could have cost him his life.

Similarly, Timoféeff-Ressovsky's behaviour must be considered in the light of the society in which he lived. Specifically, the basic questions should be answered: What was Timoféeff-Ressovsky's credo? And did he follow it?

Timoféeff-Ressovsky was deeply religious. He believed in the absolute character of good and in the transient character of evil. He considered science a humanistic force, while believing that it was not able to answer the basic moral questions.

When he lived in Germany, he helped many people to hide at the risk of his own life. His voluntary return to the Soviet Union, about which he had thought deeply, almost cost him his life: he was persecuted for having been in Germany and nearly died from pellagra in Gulag.

We wish to stress that the division of

people into "heroes and villains" is a direct consequence of existence under a totalitarian regime which (especially for active people) leaves almost no possibility of choice. Refusing to become a villain, Timoféeff-Ressovsky must have been a hero.

It was in the Soviet Union that Timoféeff-Ressovsky accomplished what was probably his most important achievement: the restoration of communication between different generations of biologists, which had been almost lost because of Lysenko.

According to the opinions of people who knew him well, Timoféeff-Ressovsky — the descendant of the princely Vsevolzhskies — never betrayed his ancestral motto: "Honour above all!"

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## Youthful virtues

SIR—In his recent Commentary article<sup>1</sup>, John Ashworth provided extremely useful data on the complex relationship between research and teaching in universities. As he points out, and as I have argued elsewhere<sup>2,3</sup>, the British government's rejection of the 'tiering' (separation) of research and teaching at the level of the institute may be countered by *de facto* forces. So it is important to examine closely what little data we have; I suggest that Ashworth's are open to an alternative interpretation.

Ashworth himself notes that, at the University of Salford, the faculty has obtained much increased research-grant support from a variety of sources, which has led to there being more postgraduates able to assist in teaching and that these students now actually do a large amount of teaching.

Further, plausible results of this arrangement are that permanent members of faculty not only can do more research, but, because they are under less pressure from teaching, can do what teaching they do better. And because the postgraduates are young and energetic and motivated to prove themselves, the teaching they carry out is especially apposite and supportive; having recently progressed through the undergraduate ranks, they will be well aware of what their students are up against. So, ironically, Ashworth's data could well be taken to support the argument for the separation of research and

teaching.

Fundamental questions remain to be answered. What is the amount and form of contact between the permanent research faculty and undergraduates, and between the postgraduate teachers and undergraduates? How (if at all) does the postgraduate involvement in research affect their teaching? The answers may tell us that youth, and recent familiarity with the needs of undergraduates, carry their own virtues. If so, that implies a very different form of tiering.

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## Window boxes?

SIR—The stimulating article by Inoue and Horikoshi (*Nature* 338, 264–266; 1989) to the effect that one may select organisms that can grow in concentrations of toluene substantially greater than those normally used to make them permeable and thereby to kill them, leads one to remark that while the mechanism of this resistance remains uncertain (although it must involve changes in outer membrane permeability), this type of ability may be quite widespread and certainly less novel than supposed. It is for instance a common laboratory observation that a 20 per cent (w/v) solution of the detergent Triton X-100 held on a window ledge will quite soon (2–4 weeks) acquire a substantial crop of green algae.

In the spirit of the age in which school-children make high- $T_c$  superconductors, this type of experiment would seem eminently suitable for more widespread adoption, especially in the light of the recent 'greening' of our politicians.

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## Free speech

SIR—Mr Salman Rushdie's right of free expression to denounce the canon of Islam has been lauded in recent weeks throughout the Western world.

Could not a similar freedom of speech be accorded to scientists to express heterodox views, or even simply to state facts that go against orthodox theory?

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# The case of the peripatetic fossils

John A. Talent

Through the activities of one Indian scientist, the palaeontological literature on the Himalayas has become shot through with disinformation.

INDIA'S Himalayan region is a paragon of mountains still being generated by collision between continents. India is also famed for its long tradition of excellence in geology and palaeontology, enshrined in more than a century's publications of its geological survey.

At first sight, an avalanche of palaeontological data published in the past 25 years would seem to have added substantially to this impressive corpus of older literature. Not only have there been biogeographically astonishing additions to the faunal lists, but revisions of stratigraphical alignments for virtually every horizon, Cambrian to Jurassic, and from one end of the Himalayas to the other. Much of this new information is based on forms never previously encountered in India or the rest of Asia<sup>1</sup>, and has considerable implications for global patterns of stratigraphy and palaeobiogeography.

Pivotal in evaluation of the new data is the saga of the Muth Quartzite and associated stratigraphical units, particularly assessment of their age, faunal content and inferred environmental setting. The Muth, an intertidal-to-aeolian unit originally discriminated in Spiti<sup>1-4</sup>, has been widely identified in Kashmir, Kinnaur, Kumaon, the Zaskar region of Ladakh and Nepal. Early in this century, poorly preserved shelly faunas<sup>5</sup> from "sandy shales, patchily calcareous" (now referred to as the Naubug Beds) underlying and transitional to the Muth in south-east Kashmir<sup>6</sup> were described; it was concluded that they were Early Silurian (Llandovery)<sup>5</sup>, an age with which a number of subsequent workers agreed<sup>1</sup>. In the 1960s, well-preserved graptolites of manifestly Late Silurian age were reported from "three closely associated horizons" (ref. 7) that were stratigraphically well below the previously described shelly faunas. Curiously, later investigators failed to find graptolites at this locality. More disconcertingly, the rocks there have been subjected to multiple deformation of such intensity as to

make it unlikely that fossils of any kind, least of all fragile graptolites, could have survived<sup>1</sup>.

In the period 1965-74, a host of papers appeared on the squashed, sheared and near-obiterated shelly fossils, said variously to be from the Naubug Beds, or "stratigraphically towards the middle of the Muth Quartzite" in south-east Kashmir<sup>8</sup>. There was no fundamental difference from the shelly faunas described earlier by Reed. The new materials, although often indeterminate as to genus, family and, occasionally, phylum, were confidently identified to genus and species levels using archaic nomenclature. This time, despite advice to the contrary<sup>1</sup>, they were repeatedly asserted to be of Devonian or Late Silurian age<sup>8,9</sup>, thus generating a large shift in stratigraphical alignments<sup>10</sup>.

In 1967 (ref. 11), conodonts came onto the Himalayan scene and assumed prime importance in a host of new correlations. What was clearly a single conodont fauna was reported from numerous places in north-east India and Nepal, sometimes from high in the Muth<sup>12</sup>, or "from just below the Muth" (ref. 13), sometimes regarded as Lower Devonian<sup>14</sup>, sometimes Middle Devonian<sup>15</sup> and sometimes Late Devonian<sup>12</sup>; the lack of consistency was striking. The fauna is in fact indicative — with high precision — of the Lower *asymmetricus* Zone, the basal conodont zone of the Late Devonian. Additionally, the fauna on which these reports was

based is an otherwise unique association, reported elsewhere only from the North Evans Limestone at Amsdell Creek, New York. The North Evans occurrence, famed throughout the 'conodont community', is an anomalous lag deposit, generated by the winnowing of materials from sources representing three or four successive conodont zones: "*varcus* (probably), *hermanni-cristatus*, *disparilis*, and Lower *asymmetricus* zones are involved in the admixture" (ref. 16). The limestone lens was quarried by Raymond Hibbard of Buffalo, New York, and samples were distributed so widely that most conodont workers of the 1960s and many universities where palaeontology is taught received copious samples of this rich and peculiar fauna.

Also disturbing is that specimens of the North Evans fauna, used for documenting an occurrence near Kishtwar<sup>15</sup> in India, were used again to argue for a similar age for a horizon on Phulchauki mountain in central Nepal<sup>17</sup> (Fig. 2). Unacceptable laboratory practice was thus confirmed<sup>18</sup>. Thorough sampling of the Phulchauki section<sup>1</sup> failed to reveal a Devonian sequence. The meagre conodont fauna that was recovered suggests a Llandovery (Early Silurian) age, compatible with previous determinations of trilobites<sup>19</sup>. Incidentally, these Silurian conodonts are from a horizon stratigraphically above a site said to have produced a specimen of the Middle to Late Devonian crinoid

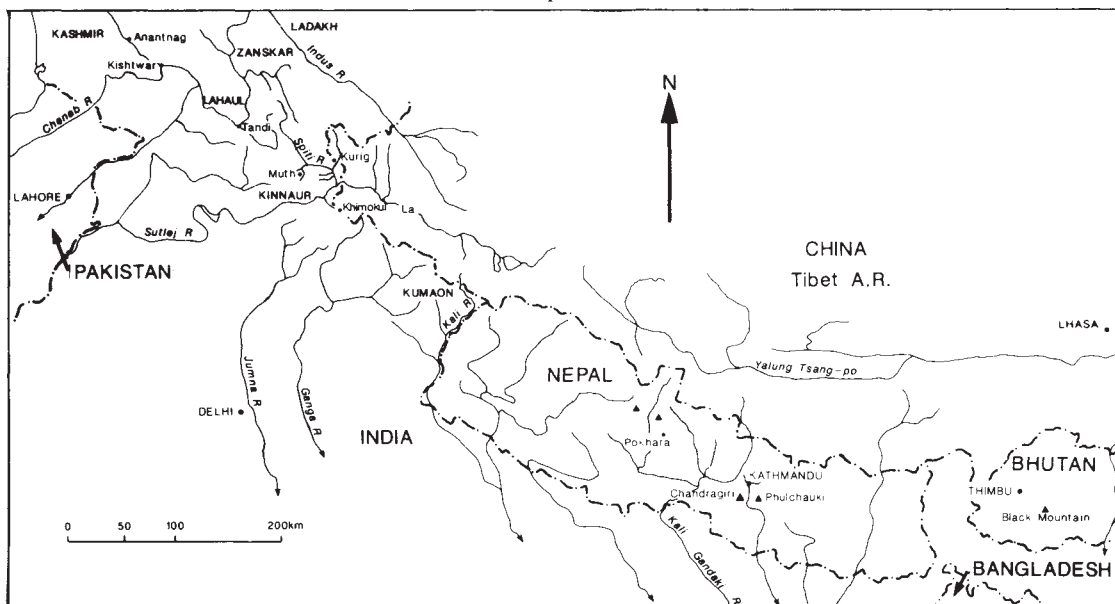


FIG. 1. Principal fossil localities in the Himalayas referred to in this article.

*Melocrinites*<sup>20</sup> — another stratigraphical anomaly. The report of Devonian conodonts, bolstered by the report of *Melocrinites*, has caused much confusion in the otherwise apparently simple stratigraphy of the ranges (Mahabharat Lekh) south of Kathmandu<sup>1,21,23</sup>.

There are numerous instances in which comparable confusion has been engendered in the stratigraphy of the Himalayas, from Kashmir to Bhutan, because of assertions of ages based on materials too poorly preserved to warrant identification, of suspect origin or not supported by illustrations. An example is the argument over strata near Tandi in Lahaul, for which conodont identifications, without supporting illustrations, were proffered in support of a Triassic<sup>24</sup> rather than a Jurassic age<sup>25</sup>. They were said to have been confirmed by Walter Sweet, the leading authority on conodonts of Triassic age, but this he has denied<sup>1</sup>.

Over the past 20 years the Himalayas have been the scene of many startling discoveries involving species, previously known in great abundance from specific places on other continents, that can be said to be 'well-fingerprinted' by highly characteristic modes of preservation, and that had long been in teaching collections around the world. Among such suspect materials is a late Famennian (latest Devonian) ammonoid fauna reported<sup>26</sup> to have come from two calcareous horizons separated by 20 metres of quartzites in the upper part of the Muth Quartzite at Khimokul La, a high pass on the Indo-Tibet border. They were said, once again, to be associated with an early Late Devonian Lower *asymmetricus* Zone ('North Evans') conodont fauna. These pelagic ammonoid and conodont faunas are both superbly constraining as to time — different times. For them to occur in association would be biostratigraphically bizarre.

More surprisingly, identical ammonoids (Fig. 3), distinctively preserved as haematite moulds and quarried as a 'cottage industry' at Erfoud, Morocco, are available world-wide from fossil dealers, rock shops and curio counters in flea markets<sup>1</sup>. The distinctive style of preservation is not known from the Himalayas, and no calcareous horizons were noted in the Muth by those who recently mapped the area and interpreted the environment as intertidal and not pelagic<sup>27</sup>; Khimokul La, as it happens, is virtually inaccessible to foreigners and most Indians other than military personnel and border guards.

A possibly parallel case is the Early Devonian ostracode fauna from an unspecified site near Kurig, close to the Spiti-Tibet border<sup>28</sup>. Conceivably, all species from this fauna could be identical with the well-known<sup>29</sup> ostracode fauna of the Early Devonian (Lochkovian) Haragan Formation of Oklahoma, but the documentation leaves something to be

desired. In view of the profound biogeographical differentiation in ostracode faunas of this age, their occurrence on the opposite side of the world is astonishing. Despite a request, when the paper was being delivered orally<sup>30</sup>, for precise locality data to enable a future generation of workers to re-collect the same horizon with some measure of confidence, such information was not given in the published paper<sup>28</sup>. Further, like the North Evans conodont fauna, Haragan microfossil washings are common in teaching collections around the world.

There are a host of similar records from the Himalayas that are palaeobiogeographically enigmatic<sup>1</sup>. If correct, these reports would indicate that the global distributional data accumulated over the past century and synthesized into palaeobiogeographical patterns has been seriously — even wildly — out of kilter for most of the Palaeozoic and Mesozoic. Alternatively one might be tempted to hypothesize the presence of numerous small, far-travelled tectonostratigraphic terrains, characterized by palaeobiogeographically anomalous faunas. There is no evidence for such anomalous entities stitched into the otherwise stratigraphically simple, Palaeozoic–Jurassic passive continental margin sequence along the northern flank of the Indian continental block, south of the Indus–Yarlung Tsangpo Ophiolite Belt. Lithofacies and biofacies tend to be remarkably persistent, often over hundreds of kilometres, paralleling ancient shorelines. The palaeozoogeography for any specified interval of time is therefore simple, aside from the recent deluge of anomalous reports.

Similar cases of carelessness over data or confusion over concepts are rife. Biozones have been proposed without evidence of an understanding of the craft of biozonation, named from long-ranged or problematic genera and dubious or misidentified species, or even for lithological intervals lacking the requisite 'bio' (ref. 31). It is not clear how such entities might be aligned with international zonal schemes.

It is also puzzling that manuscripts describing these finds were submitted for publication subsequent to and reporting — without referring to the similarities, either in footnotes or postscripts — data virtually identical to that in manuscripts made available earlier in pre-publication copies distributed at scientific meetings<sup>1,32–34</sup>. In each case a co-author of the subsequent paper was in attendance.

Moreover, it is rare to find anyone credited with having collected any of the palaeobiogeographically unexpected items that have been reported from the Himalayas in the past 20 years. Nonetheless, there is one name associated with each of these remarkable discoveries. Cambrian to Cenozoic, protists to pri-

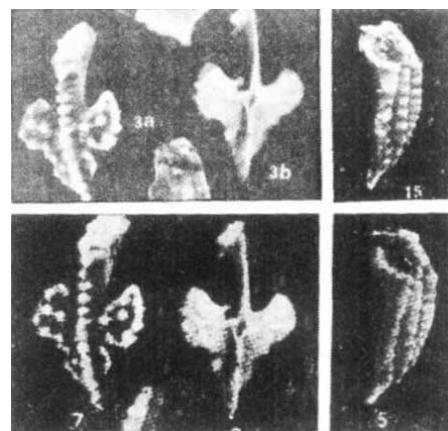


FIG. 2 Late Devonian conodonts said to be from Kishtwar, India<sup>15</sup> (above), but also said to be from Phulchauki, Nepal<sup>17</sup> (below), cut from the original publications.

mates and land plants, reported in scores of papers in an *oeuvre* of more than 300 publications spanning the whole gamut of things geological published in the past two decades. Quality of publications aside, this is a remarkable performance.

However that may be, the database for the Palaeozoic and Mesozoic of the Himalayas has, as a consequence of these publications, become so marred by inconsistency as to throw grave doubts on the scientific validity of any conclusions that might be drawn from it. Because the biostratigraphical underpinning of so much Himalayan stratigraphy is in question, the credibility of many years of labour by numerous geologists is at stake. The question at issue can be answered only by confirming the reproducibility (or otherwise) of the data by collection of *in situ* specimens, of the species judged to be biostratigraphically and biogeographically bizarre, from precisely the same horizons and places in the Himalayas from which they have been reported. Given the nature of the locality information provided, this may be no easy task. There are plenty of examples where incorrect or unhelpful information has been given<sup>12,28,31,32,35,36</sup>, often by coordinates of towns or villages kilometres away from the localities themselves<sup>1</sup>. One of these 'localities' (ref. 37) was 130 kilometres or so of Himalayan valley walls.

It is possible to construe the evidence as consistent with a concatenation of curatorial disasters — mislabelling, accidental contamination, peripatetic locality labels — but this would have to have occurred on a monumental scale. Whatever the cause or causes, numerous Indian scientists were 'stung' by having their names associated with papers on materials of dubious provenance. More than a score of foreign scientists, having been requested to confirm identifications and having done so, have been made co-authors of papers in which, sometimes, surprise would be expressed at the associations and even at the quirks of preservation being indistin-



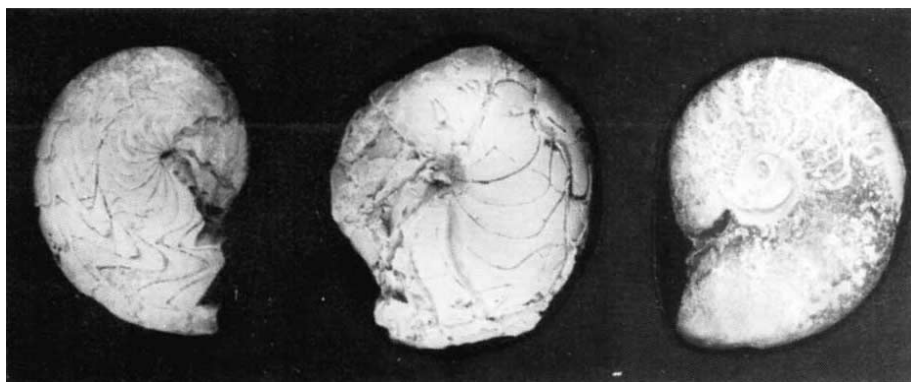


FIG 3. Ammonoids preserved as haematite moulds, from the vicinity of Erfoud, Morocco<sup>1</sup>, typical of material available worldwide from fossil dealers (these examples purchased from Alain Carion, Minéraux Fossiles, 92 rue St Louis en l'Île, 75004 Paris). For comparison with material reported as Indian from the IndoTibet border at Khimokul La<sup>26</sup>.

guishable from materials from places virtually at the experts' back doors<sup>38</sup>. Doubtless this was, in fact, often the ultimate provenance of the materials in question. Verification both of the fossil occurrences and the associated stratigraphy and biostratigraphy, preferably by an independent commission<sup>1</sup>, is needed — a herculean task. In the meantime, it seems prudent to ignore all publications bearing the name of the author associated with all the dubious reports identified here.

Sadly, the dubious data and the stratigraphical conclusions based on them are being treated as reliable for global (for example, see data points in ref. 39) or semi-global palaeogeographical and stratigraphical syntheses<sup>40</sup>, and are being incorporated into otherwise commendable textbooks<sup>41</sup>. A fair amount of the information has also been stitched into a five-volume treatise on Indian stratigraphy<sup>10,31,42-44</sup> which the unsuspecting could take to be authoritative. This series should have updated and therefore superseded Sir Edwin Pascoe's exhaustive *Geology of India*<sup>45</sup>, but successive volumes were more haphazard as to what new data were included, and the handling of the information became increasingly cavalier and decreasingly dependable. It would therefore be perilous to use these volumes for understanding the geology of the subcontinent or as data sources for inter-regional or global palaeobiogeographical or tectonostratigraphical analyses. Immeasurably safer would be to return to Pascoe and build from there.

How is it that so much material of suspect origin has survived the scrutiny of the scientific world for so long? How, for instance, have so many biostratigraphically and palaeobiogeographically unlikely conodont-ammonoid, brachiopod-brachiopod<sup>26</sup> and conodont-conodont associations slipped through the refereeing process? Why has so much obviously unidentifiable material been so freely endowed with generic and specific names which were allowed to get into print and accepted as trustworthy? Why have so

many novel stratigraphical alignments, based on little more than assertion, been allowed to go unchallenged?

Science in India, as elsewhere, has yet to find ways of reducing incentives to publish, for example, by laying less stress on quantity as the primary basis for gaining continued support and accelerated promotion. It is even more difficult to develop mechanisms for dealing with the major crimes of data fabrication and plagiarism, and the minor ones of sloppy reporting, honorary authorship, giving results in minimal units and laying false trails to deceive competition. Clearly, the eagerness of publishing houses to profit from scientific publication and the trust, even reverence, accorded to scientists by Indian publishers has contributed to the

spate of volumes dealing with Himalayan topics.

Most scientists are reluctant to 'blow the whistle' on a colleague; and most tend to have highly specialized research interests. So even if a specific item of data is suspected of being false, it would directly affect very few people. The tendency is to dismiss such episodes as the one-off aberrations of scientists straying outside their accustomed bailiwicks, rather than as elements of a potentially much larger fabric: in the present case an especially vast and intricate fabric.

Rhinos in Rio? Kangaroos in Kashmir? Well, something as remarkable biogeographically is said to have occurred. At first sight it might appear that a whole circus of exotica — mainly invertebrate — was let loose and fossilized seriatim in the Palaeozoic and Mesozoic sequences of the Himalayas. Earth scientists in general, and palaeontologists in particular, have blissfully assumed that, apart from the Piltdown Man, their science was largely free from attempts to pollute the literature. There have been cases of practical jokes, and examples of misappropriation of materials by individuals over-eager to publish. But compared with the cornucopia of items disgorged into the stratigraphy of the Himalayan region over the past 25 years, such instances are mere bagatelles. □

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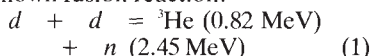
# Consensus on cold fusion still elusive

Accounts of the cold-fusion experiments at the University of Utah and Brigham Young University were presented last week at a meeting at the Ettore Majorana Centre for Scientific Culture, but many questions remain unanswered.

## Erice, Sicily

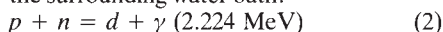
AFTER a full day of presentations and discussion of the recent claims of cold nuclear fusion, there was no consensus at this meeting on the results, no credible theory to explain them, but some suggestions as to where to look for an explanation or confirmation. The work of the Utah group<sup>1</sup> was presented at the meeting last week by M. Fleischmann, that of the Brigham Young group<sup>2</sup> by S.E. Jones and J.B. Szirr.

The details of the experiments are important in any comparison or assessment. Jones and his colleagues electrolyse heavy water (D<sub>2</sub>O in a solution at pH 3 of a witches' brew of salts (including Li and Pd) with Pd foil on rough Ti or Pd chunks as cathodes, driving deuterium into the metal with a voltage of 3–25 V and cell currents of 10–500 mA. In each 20-ml cell, the anode is gold foil. A counter designed to detect and identify fast neutrons indicates a total of  $170 \pm 23$  counts with a pulse-height spectrum consistent with that expected for the 2.45 MeV neutron of the well-known fusion reaction:



J.B. Szirr described the neutron detector in detail. It detects a thermalized neutron by the light flash in 'Li-doped glass'. The neutron energy is determined by the overall fast light-pulse caused by protons recoiling in a liquid scintillator as the neutrons are thermalized. The counting rate is but 2 per hour in the relevant region of pulse height.

The analogous experiments<sup>1</sup> by Fleischmann, Pons and Hawkins use a strongly alkaline solution of 0.1 M LiOD in heavy water and drive deuterons into Pd rod cathodes (cast and machined) under the influence of cell currents up to 800 mA and voltages typically of 12 V. The plan is to detect reaction (1) by the 2.22 MeV  $\gamma$ -ray resulting from capture of the 2.45 MeV neutron (after thermalization) by a proton of the surrounding water bath:



The authors describe a very narrow peak at 2.2 MeV containing some 3,000  $\gamma$ -ray counts for an NaI scintillation detector close to the electrolytic cells, in comparison with the 'level spectrum' in a similar detector 5 m

or 10 m away. Unfortunately, the full pulse-height spectrum is not shown, so that it is not possible to verify the presence of the annihilation radiation 'escape peaks' that would lend more confidence to the origin of these counts in the neutron-proton interaction (2). Furthermore, no evidence has been given which connects the counts with current applied to the cell, and there have been no runs with ordinary water as a control.

In the scientific and patent literature over the past 60 years, there have been occasional claims of nuclear fusion catalysed by palladium, but there have previously been no credible reports of neutrons from metal deuterides, for reasons thought to be well understood.

Briefly, these are that the Coulomb barrier to close approach by nuclei of charges  $Z_1e$  and  $Z_2e$  amounts to  $(Z_1e)(Z_2e)/r$ , where  $r$  is the nuclear diameter. This amounts to about 600 keV when  $Z_1 = Z_2 = 1$ , reducing to a very small value the probability that, by quantum mechanical tunnelling, the deuterons in a D<sub>2</sub> molecule will approach within the range of nuclear forces.

Even so, the calculation of the rate at which deuterium nuclei in a molecule will undergo fusion is important as a yardstick for assessing the rates reported in the recent experiments. At last week's forum, new calculations were presented<sup>3</sup> by S.E. Koonin (Santa Barbara) of the number of fusion reactions per deuteron bound in a deuterium molecule by 'electrons' of normal charge but mass  $m^*$  instead of  $m_e$ . If the logarithm (base 10) of the number of fusions per deuteron per second is  $\lambda$  (with a subscript to indicate the fusion mode), the results come out as follows:

|                | Log <sub>10</sub> of fusion rate per $d$ per second |      |      |      |
|----------------|-----------------------------------------------------|------|------|------|
| $m^*/m$        | 1                                                   | 2    | 5    | 10   |
| $\lambda_{dd}$ | 63.5                                                | 40.4 | 19.8 | 9.1  |
| $\lambda_{pd}$ | 55.0                                                | 36.0 | 19.0 | 10.4 |

Others at the meeting agreed with these results, which correct an error in some previous calculations and use a more accurate molecular potential. An important experimental point is provided by the case in which  $m^* = 207$ , corresponding to that in which a negative muon binds two deuterons as a molecular ion 207 times smaller in dimensions than the normal molecular ion,

with  $\lambda_{dd}$  of some  $10^9 \text{ s}^{-1}$ , as predicted<sup>3</sup> and observed<sup>4</sup>.

These results also show the strikingly easier penetration of wide barriers by the proton. The explanation lies in the sensitivity of the chance that the Coulomb barrier will be penetrated by quantum mechanical tunnelling to the "reduced mass"  $\mu$  of the two nuclei, which is  $M_1M_2/(M_1 + M_2)$ . Numerically, the barrier penetration factor is  $e^{-2 \int k(r)dr}$ , where the integral runs from zero to the classical turning point  $r_0$  and the function  $k(r)$  is  $[2\mu(V(r) - E)]^{1/2}$ .

The rate of neutron production claimed by Jones *et al.* is  $\lambda_{dd} = 10^{30}$ , which would require that  $m^*/m_e$  was equal to 5, according to the figures in the table. A similar value of the effective mass is needed to explain the  $\gamma$ -ray counts reported by Fleischmann and Pons.

Although quasi-particles of high effective mass are well known in metals, the value of  $m^*$  relates to the relationship between the density of states and the energy in the band structure of the lattice. Thus a quasi-particle of effective mass 5 is not capable of binding two deuterons to a density  $5^3$ , or 125, times that of molecular hydrogen, or of allowing the nuclei to approach one another to a distance 5 times smaller than the 0.74 Å internuclear separation in the D<sub>2</sub> molecule; at this distance, 0.15 Å, the repulsive potential amounts to some 95 eV.

That is why it was argued at the forum last week that one should look for dynamic effects to augment the equilibrium tunnelling — phonon-assisted tunnelling (Koonin) or coherent acceleration (Ponomarev, USSR) in which travelling electron density waves may trap deuterons and accelerate them to the same velocity as

the waves. The deuteron kinetic energy would then be some 3,700 times that of an electron of the same velocity and would greatly enhance the chance of penetrating through the barrier by quantum tunnelling. Alternatively, a solution could be sought in "high- $T_c$  superconductivity or other miracle of solid-state physics".

Several experimental groups at the forum

*The forum on cold fusion held on 12 April at the Ettore Majorana Center was convened by Professor Antonino Zichichi, director.*

presented results showing no neutrons or  $\gamma$ -rays generated in replication of the experiments which have been described<sup>1,2</sup>. Electrolysing 1-mm by 10-cm Pd rods for 10 days gave neutron yields below  $0.6 \text{ s}^{-1} \text{ cm}^{-3}$  (M.M. Broer, AT&T Bell Laboratories) or less than  $10^3$  of those reported by the Brigham Young group in similar circumstances. J. E. Ziegler (IBM) reported an upper limit of  $10^3 \text{ s}^{-1} \text{ cm}^{-3}$  for the detection of  $t$  or  $p$  from the  $d+d$  reaction. Experiments were reported (Celani, Frascati) with "some increase in neutron signal at the beginning of each experiment for about 5 minutes, but indistinguishable from background after 20 minutes". Experiments will be transferred to the great underground laboratory at Gran Sasso; perhaps also those who claim the ability to produce neutrons will be hospitable to those more adept at detecting than at producing them in this way.

*Non l'avrei giammai creduto... Ma farò qualche potro...* ("I would never have believed this, but I'll see what I can do", says Mozart's *Don Giovanni*, quoted by L. Maiani).

This, of course, refers to the heat generation claimed<sup>1</sup> of some  $10 \text{ W per cm}^3$  for 100 hours or more, as well as destructive releases of heat that fuse and vaporize Pd and destroy the cell. The most likely explanation of such violent happenings is that they are the result of the electrochemical creation of high explosive by stuffing hydrogen into high-energy sites in Pd (analogous to the Wigner energy in neutron-irradiated graphite). But no such explanation can account for the  $4 \text{ MJ/cm}^3$  (or  $600 \text{ eV}$

$12 \text{ V}$ ). Stored energy could be at most  $3 \text{ eV}$  or so in any chemical reaction, so it is of the utmost importance to enquire into the details of this measurement; unfortunately, details are lacking.

The 'excess enthalpy generation' is measured calorimetrically by a "calibrated thermistor" as  $\Delta T$  between the (sometimes stirred) contents of the electrolytic cell and a surrounding thermostated water bath, in comparison with the  $\Delta T$  measured for a resistance heater in the cell; the thermal impedance is that posed by the dewar flask in which each experiment is conduc-

$\text{s}^{-1}$  is the accompanying claim to have detected the production of only  $4 \times 10^3$  neutrons per  $\text{cm}^3$  per second. This means that fewer than 1 in  $10^9$  of the reactions are supposed to produce a neutron.

How can this happen? A coherent superposition of isotopic spin states for  $d+d$  could cancel out the neutron-producing reaction and reinforce the  $t+p$  branch (D. Wilkinson, Sussex), but would not eliminate the usual isospin-zero reaction. It would thus change the neutron branching-ratio only if the barrier penetration were greatly facilitated. This effect can be estimated, and

**... a multi-dimensional revolution. I bet against its confirmation.**

ted. For rods of 1-mm, 2-mm and 4-mm diameter, an excess heat rate is found<sup>1</sup> that depends strongly on the current density, amounting to  $8\text{--}20 \text{ W/cm}^2$  at  $0.5 \text{ A/cm}^2$ , which excess heat persists during the operation of the cell for hundreds of hours, even though the surface of the Pd rod blackens.

I have seen insufficient evidence to believe that there is 'excess heat', since the  $\Delta T$  is measured between the bath and the

I judge the effect is only a few per cent rather than a factor  $10^9$ .

Suggestions of radiationless deexcitation of the  $^4\text{He}$  intermediate state formed by  $d+d$  thus far fail in two regards: first, the lack of a mechanism and, second, because such a mechanism would add a channel to the usual particle decay of  $^4\text{He}$  rather than suppress the usual channel. Thus such a mechanism would need to be  $10^9$  times faster than the usual particle channels that themselves occur in nuclear transit times — a totally new phenomenon. Finally, the needed mechanism must not have shown itself in the measurement of the cross-sections — some of which were done in gas cells, but some, at times, in metal hydrides.

*Somebody is going to have to eat his hat* (L. Maiani).

*We are also human, and need miracles, and hope they exist.* (L. Ponomarev, Moscow).

A few neutrons each second (or a few thousand) from an electrolytic cell may be cold nuclear fusion or may have an 'arcs and sparks' origin. Within the next few weeks, experiments will surely show whether cold nuclear fusion is taking place; if so, it will teach us much besides humility and may indeed provide insight into significant geophysical puzzles<sup>3</sup>. Large heat release from fusion at room temperature<sup>1</sup> would be a multi-dimensional revolution. I bet against its confirmation.

**Richard L. Garwin**

**... experiments will show whether cold fusion is taking place; if so, it will teach us much besides humility ...**

per atom) to which continuous energy release at such a rate would correspond.

One issue to be checked is whether there is indeed any such excess heat flow to the surrounding water bath, in excess of that represented by the product of current and voltage applied to the cell (typically  $0.8 \text{ Å}$  at

thermistor in the electrolytic cell itself, rather than along a fixed conductive link between cell and bath. If there are significant temperature gradients within the cell because of imperfect stirring or local recombination of hydrogen and oxygen gas, the thermistor temperature will not be the temperature of the inside wall of the flask, resulting in very substantial errors in inferred heat generation.

Even more striking than a heat-producing fusion reaction at a rate some  $6 \times 10^{12} \text{ cm}^{-3}$

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# Drosophila back to front

Ken Howard

WHY does the fate of a cell depend on its position in the embryo? One simple mechanism involves diffusion of a morphogenetic molecule from a localized source to form a concentration gradient. The morphogen concentration, which depends on distance from the source, controls gene activity and so determines cell fate. A mechanism of this kind is known to underlie the specification of the anterior segments of the *Drosophila* embryo by the product of the *bicoid* gene early in embryogenesis<sup>1-4</sup>. *Bicoid* messenger RNA is localized at the anterior of the egg during oogenesis and provides a localized source of the protein product in the early embryo. Changes in the concentration profile of the *bicoid* protein produce corresponding changes in the positional value, and when embryos lacking *bicoid* maternal mRNA are rescued by injection of cytoplasm containing this RNA into the egg, the head end is always defined by the site of injection (see figure). The posterior segments of the embryo depend on another gene, *nanos*, which produces a factor localized at the posterior end of the egg<sup>5,6</sup>. In the absence of the *nanos* product, the posterior segments (roughly equivalent to the abdomen) do not form. A concentration gradient of *nanos*, however, does not specify position in the posterior. On pages 633 and 650 of this issue<sup>7,8</sup>, and in a paper to be published next week<sup>9</sup>, Hülkamp *et al.*, Irish *et al.* and Struhl report experiments showing that the sole function of *nanos* is to prevent expression of a third gene, *hunchback*, in the posterior of the embryo.

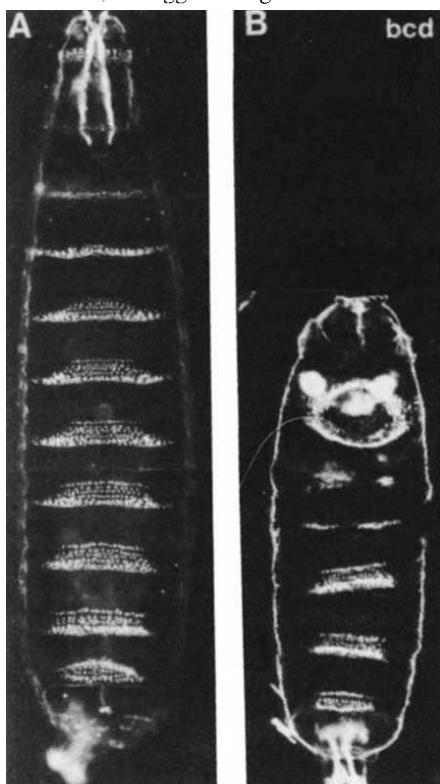
*Hunchback* belongs to a group of genes called gap genes, because large continuous regions of the body are missing from larvae with mutations in those genes. What the experiments with *nanos* mutants now suggest is that position in the posterior segmental pattern of the fruitfly is determined by local interactions between the gap genes, and no long-range morphogenetic signal is required.

Although *bicoid* and *nanos* (and other genes determining the ends of the embryo) are transcribed maternally so there is no requirement for the gene itself for embryogenesis, the gap genes, with one exception, are activated only in the zygote. The exception is *hunchback*, which has maternal as well as zygotic transcripts. Maternal *hunchback* mRNA is uniformly distributed throughout the egg<sup>10</sup>, although it seems to have no function: flies will develop normally from an egg lacking the maternal *hunchback* mRNA provided that the zygote contains a normal *hunchback* gene<sup>11</sup>.

What Hülkamp *et al.*<sup>7</sup> and Struhl<sup>9</sup> now

show is that not only is the maternal *hunchback* product unnecessary for normal development, but normal development of the posterior cannot proceed in its presence. Tautz has earlier shown that *nanos* represses the translation of the *hunchback* mRNA in the presumptive abdominal regions of the embryo<sup>12</sup>; Hülkamp *et al.*<sup>7</sup> and Struhl<sup>9</sup> now demonstrate that when *hunchback* protein is produced here, abdominal development is disrupted. When it is produced at relatively high levels under the heat-shock promoter, phenotypes very similar to that of the *nanos* mutant result<sup>9</sup>.

How does the ectopic *hunchback* protein interfere with normal posterior development? One possibility is that it represses other genes that normally respond to a gradient of *nanos* product. In this case, an egg lacking both the *nanos*



A, Wild type *Drosophila* embryo; B, strong *bicoid* mutant, where the head and thorax are replaced by a duplicated telson, and the anterior abdomen is defective. Anterior is at the top. (From ref. 3.)

factor and *hunchback* mRNA should fail to develop the posterior pattern because although there would be no early *hunchback* protein to suppress the response to the *nanos* gradient, there would also be no *nanos* gradient to respond to.

In fact, the phenotype of the double mutant is far more interesting. Using different techniques to make eggs without either maternal *hunchback* or *nanos*

product, all three groups<sup>7-9</sup> show that in the absence of maternal *hunchback* mRNA the *nanos* factor is unnecessary. Viable flies develop from the double-mutant eggs when they are fertilized by sperm containing a wild-type copy of *hunchback* to provide the zygotic activity necessary for normal development.

If it is not a gradient of *nanos* factor, then what does tell the cells in the posterior of the embryo where they are? One possibility is that there is another morphogenetic factor localized in the posterior which has yet to be identified genetically. An exciting alternative is that the information is generated by zygotic gene activities.

The first zygotically active segmentation genes are gap genes. They are expressed in broad bands along the anteroposterior axis and provide positional information that is interpreted by a second set of segmentation genes, the pair-rule genes, which are expressed periodically and set up the true segments. Mutants lacking the main gap gene involved in the posterior, *knirps*, have phenotypes similar to, but less extreme than, those lacking *nanos*<sup>5</sup>, and *knirps* expression is abolished in mutants lacking *nanos*<sup>13</sup>. Other zygotic genes must be involved in the *nanos*-dependent pattern<sup>5</sup>. At least three other gap genes, *knirps-related*<sup>14</sup>, *hunchback*<sup>10</sup> and *giant*<sup>15</sup>, and another segmentation gene, *caudal*<sup>16,17</sup>, are expressed in parts of the appropriate region of the embryo.

Although these gap genes might be passive intermediates between a maternal gradient and pair-rule genes, merely presenting the information contained in the gradient to the pair-rule genes, they could play a more active role in generating pattern. Systems consisting of components that diffuse and affect each other's rate of production can spontaneously develop spatial patterns<sup>18</sup>. The final pattern is influenced by the initial conditions, but is largely determined by the inter-

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actions themselves. Consequently, these reaction-diffusion systems can show self-organizing ability.

Interactions between *knirps* and its anterior neighbour, *Krüppel*, have already been described<sup>19</sup>. It will be interesting to see how the posterior gap genes regulate one another and if it is possible to explain the formation of the posterior pattern as a consequence of these interactions.

Two sets of observations are consistent with this sort of mechanism. First, the posterior system has self-organizing properties which contrast with the anterior *bicoid* system<sup>4</sup>. Most dramatically, *nanos* factor injected into the middle of an embryo which lacks both *bicoid* and *nanos* induces abdominal segments radiating from the poles of the embryo, not the site of injection (*bicoid* activity induces anterior

segments radiating from the site of injection). This difference may be due to the properties of the zygotic genes involved in posterior patterning<sup>4</sup>.

Second, the posterior pattern can respond to changes in zygotic gap gene activity. For example, Lehmann has shown that *pumilio* mutants, which prevent diffusion of the *nanos* activity, can be suppressed by mutants in the terminal zygotic gap gene *tailless*<sup>20</sup>.

The present work raises many questions, above all, what determines position in the posterior region? Is there an undetected maternal gradient? Do gap-gene interactions spontaneously generate the pattern, or is something else going on? □

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## METEOROLOGY

# It never rains, but it pours

Ian N. James

SUPERFICIALLY, it can seem that the character of the weather persists in one mode for considerable periods before switching to some very different mode. For example, the early part of this winter was remarkable dry, warm and settled in western Europe. Since mid-February, an equally persistent unsettled regime associated with a sequence of vigorous depressions has dominated the weather (compare the adage in the title). Such

over mountain chains. There is much less scope for such forcing in the Southern Hemisphere, suggesting that present theories of bimodality may be inadequate.

Hansen and Sutera examined a series of daily 500-millibar geopotential height fields, analysed by the European Centre for Medium Range Forecasts, for a period of 4.5 years over the Southern Hemisphere. The greatest amplitude is associated with zonal wavenumber 3 (that is,

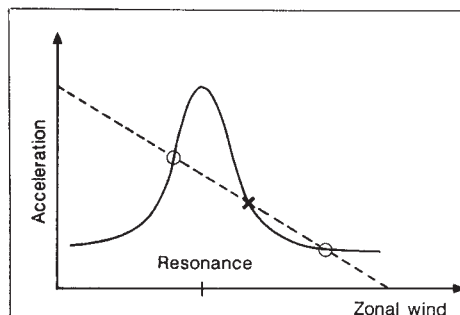
low-amplitude wavenumber 3 was present reveals a clear switching from zonal wavenumber 1 to zonal wavenumber 3.

Such bimodality in atmospheric data seems to be a hemispheric rather than a local phenomenon. Theories have been developed (see box) which predict bimodality using simple models. The crucial ingredients in such models are: first, a source of planetary-scale Rossby-wave activity in the middle latitudes, generally provided by flow over large-scale mountain ranges; and second, a resonance in the response of the large-scale flow to such forcing.

In the Southern Hemisphere, it is not clear that mountain topography can generate the observed wavenumber 3 (ref. 3). The Andes, the only considerable mountain range of the southern mid-latitudes, force a response which is confined to the subtropics. An alternative source of wave activity, perhaps associated with variations of sea surface temperatures with longitude, must be sought. A more serious problem is the need for resonance. This can occur if the zonal flow acts as a waveguide, confining the wave activity to the mid-latitudes. A wave train radiated from the source will eventually propagate back into the source region. If the waves have the right phase, resonance occurs. In the simple theories, a guide is simply provided by confining the flow with rigid boundaries to north and south; these reflect Rossby waves. But no such reflecting boundaries exist in the real atmosphere. Indeed a fundamental property of the global circulation is the largely one-way dispersion of Rossby waves from mid-latitudes into the tropics.

It is intriguing that a weak waveguide behaviour for just wavenumber 3 might be possible between 50° S and 70° S (refs 3 and 4). But such a waveguide would be extremely leaky and unlikely to generate the sharp resonance needed to explain the observed bimodality. For us to understand these observations more satisfactorily, it is necessary to develop a theory of multiple equilibria which does not depend on the presence of reflecting boundaries to the flow domain. In a system as highly nonlinear as the atmosphere, it would be no surprise to find that multiple equilibria are possible. The challenge is to find a specific mechanism. □

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stable; X unstable) if this wave drag balances the relaxation to the undisturbed state; the existence of multiple equilibria depends upon the sharpness of the resonance shown in the wave-drag curve. The introduction of weak nonlinearity can 'bend' the resonance peak, so that multiple equilibria with the same zonal wind are possible<sup>6</sup>.

superficial impressions are borne out by more careful analysis of atmospheric data. There is evidence that the amplitude of planetary-scale 'Rossby' waves in the Northern Hemisphere can switch from a larger to a smaller amplitude mode and back<sup>1</sup>. Hansen and Sutera<sup>2</sup> now suggest in the December issue of the *Journal of Atmospheric Sciences* that similar bimodality is present in the Southern Hemisphere. Understanding this result is important, because theories of bimodality rely heavily on the generation of planetary waves as the mid-latitude westerlies rise

three maxima and minima around the latitude circle) in the middle latitudes. The authors calculated the probability density of different wavenumber 3 amplitudes after removing any long-term trends. For the winter, there was a clearly bimodal distribution, whereas there was only a single maximum in the summer. Examination of the time series shows that each amplitude phase could persist for periods of 20–30 days. For other times, the authors note a more irregular fluctuation of amplitude. Combining the fields for periods in which the large-amplitude or

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# A walk in the Lyman- $\alpha$ forest

John K. Webb

THE absorption lines seen in the spectra of distant quasars are produced by matter intersecting the line of sight, and show how matter is distributed in the Universe up to the large distances of the quasars themselves. Recent work on quasar absorption lines raises important issues concerning this distribution. A. Crofts, in a paper in the *Astrophysical Journal*<sup>1</sup>, now presents observations of a group of four quasars which lie close together in the sky. In comparing the spectra of these objects, it

numerous narrow absorption lines, most of which are also Lyman- $\alpha$  at redshifts less than that of the quasar. This is the so-called Lyman- $\alpha$  forest, the subject of the interesting but controversial new paper by Crofts<sup>1</sup>. Elements heavier than hydrogen do not create similar forests of absorption lines. In fact, generally there are no heavy-element absorption lines at redshifts corresponding to individual Lyman- $\alpha$  clouds. This may be because either the gas giving rise to the absorption has low or

even primordial abundance, or simply because the amount of heavy elements present is insufficient to produce detectable absorption.

Much less frequently, there are absorption systems which clearly do exhibit heavy-element transitions (such as C II, C IV,

Si II and Si IV). The gas which produces these systems has evidently already undergone considerable chemical processing. What is not clear is the relationship between these systems and those comprising the Lyman- $\alpha$  forest. Opinion seems divided between two possibilities. Some people take the view that the Lyman- $\alpha$  clouds and the heavy-element systems all belong to the same population of objects, there being no clear demarcation in any of the observable properties. This theory is supported by evidence recently put forward, based on the claim that the distribution of neutral-hydrogen equivalent widths for both types of absorbers merge into a single power law<sup>3</sup>. This claim has not gone undisputed<sup>4</sup>. Others maintain that the two types of systems are physically very distinct. Supporters of the latter view generally associate the Lyman- $\alpha$  forest

absorbers with intergalactic gas clouds of galactic dimensions, confined by the pressure of the intergalactic medium and possibly having primordial composition<sup>5</sup>.

In his attempt to address this problem and also to explore the spatial distribution of high-redshift matter, Crofts has observed four quasars lying fairly close together in the plane of the sky — within a circle 440 arcseconds in diameter. The angular separation of these quasars means that the region of space through which the sight lines pass can be sampled on scales of approximately 0.5 megaparsec (1 megaparsec =  $3 \times 10^{22}$  m). In doing this, it seems that there are correlations in the positions of the Lyman- $\alpha$  absorption lines in each spectrum. This indicates that the Lyman- $\alpha$  clouds are clustered in space, a feature which had been recognized recently in single sight lines<sup>6</sup>, but not with adjacent quasars.

Searches for clustering of the Lyman- $\alpha$  lines along single lines of sight in earlier surveys were negative when using data with spectral resolution comparable to that used by Crofts<sup>4,5,7</sup>. Such attempts were hampered by the fact that one could never explore clustering on scales below a value determined by the spectral resolution, signal-to-noise ratio and analysis techniques. It was not until higher-resolution data were collected that some departure from a uniform cloud distribution was detected. One enormous advantage of quasar pairs or groups over single quasars is that when looking for cross-correlations, the resolution problem is not important and one can explore clustering down to much smaller scales.

The clustering properties of the heavy-element systems have been studied using the C IV absorption lines as identifiers<sup>8</sup>. It was immediately apparent that these systems are indeed very strongly clustered. Because the heavy element systems are strongly clustered, and the Lyman- $\alpha$  forest is much less clustered, it had seemed that the two kinds of gas clouds come from different populations.

Now, however, according to Crofts, the degree of clustering found when cross-correlating Lyman- $\alpha$  lines seen in

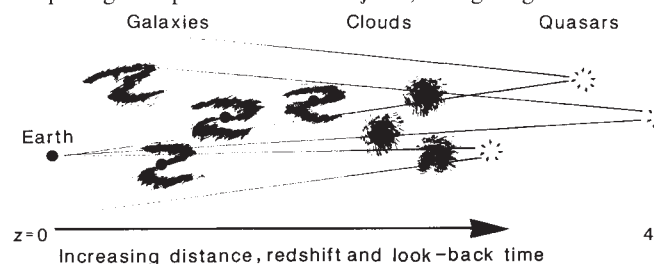


FIG. 1 Because of the cosmological expansion of the Universe, the most distant objects are those that are receding most quickly, and spectral lines are consequently strongly redshifted to longer wavelengths. Also, because of the time taken by light traversing the Universe, these objects are seen as they were at earlier epochs.

seems that correlations exist between the four quasars in the positions of the numerous hydrogen absorption lines (caused by intervening gas clouds), indicating that these objects are significantly clustered. Crofts makes the additional claim that the clustering amplitude depends on absorption-line strength. If correct, this points towards a unified picture of high-redshift absorbing clouds as a continuous population of objects.

The study of quasar absorption lines is an area of intense research and controversy. Immediately after the discovery of quasars, it was realized that they offered great potential as probes of the objects (galactic halos, intergalactic gas clouds) which lie between them and us. By studying the absorption lines imposed on the quasar spectra by intervening objects intersecting the line of sight, important information about the relatively young Universe can be obtained (Fig. 1). The current maximum redshift ( $z$ ) we can feasibly study<sup>2</sup> is just over 4. At this epoch, we are exploring the Universe when it was only about one-tenth of its present age. In principle, we can study all the way from  $z = 0$  out to these high redshifts and actually see the Universe evolving, although with ground-based telescopes the atmosphere severely restricts our wavelength (and hence redshift) window.

The most frequently detected absorption features are, not surprisingly, those of hydrogen, the most abundant element. At wavelengths below that of the quasar Lyman- $\alpha$  emission hydrogen line we see

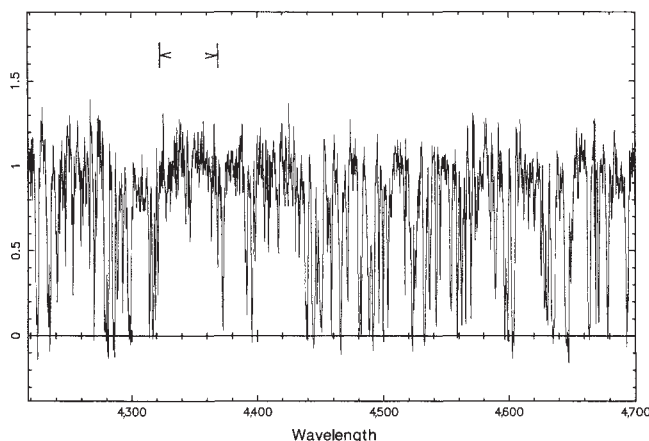


FIG. 2 A portion of the spectrum<sup>14</sup> of the quasar QSO0420-388 showing the 46-Å range around 4,350 Å devoid of absorption features, identified by Crofts. Is the apparent absence of objects at  $z = 2.58$  real, and if it is, is it representative of the structure of the young Universe?



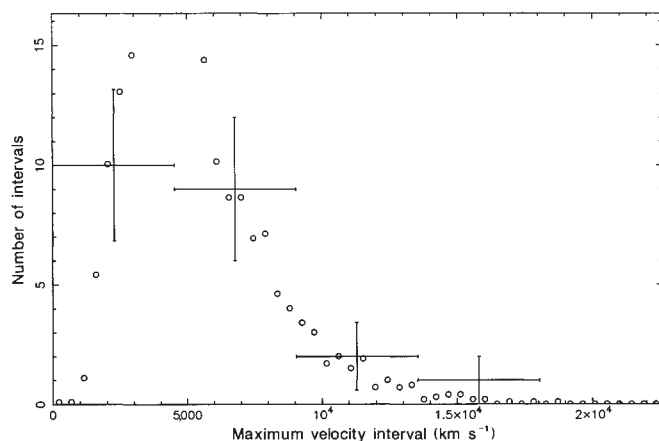


FIG. 3 A search for voids in the distribution of Lyman- $\alpha$  clouds illuminated by quasars other than QSO0420-388. Crosses, observed maximum gap sizes in 22 spectral ranges from a sample of 20 quasars (vertical bars, approximate errors; horizontal bars, range for each data bin). Circles, estimated distribution for randomly scattered hydrogen clouds, derived empirically from a large set of numerically simulated quasar spectra.

adjacent quasars depends on the absorption-line strength (rest-frame equivalent width). If correct, this would point to a continuity between the heavy-element systems and the Lyman- $\alpha$  forest, as neutral-hydrogen column densities for the heavy-element systems are generally higher than those of Lyman- $\alpha$  forest objects. From the observed Lyman- $\alpha$  cloud number density, it is inferred that these objects are far more numerous than the bright galaxies we see more locally. An attractive possibility is to associate the Lyman- $\alpha$  clouds with lower-mass objects (such as dwarf galaxies) which give a more representative view of the overall matter distribution in a Universe comprising mainly dark matter.

The observations could then fall rather neatly into the biased-galaxy cosmology<sup>8</sup>. In this picture, the most massive objects (giant galaxies) form around the sites of highest density in the early Universe, and hence are more strongly clustered than the underlying matter. If we then associate the dwarf galaxies with lower density perturbations<sup>9</sup>, we expect them to follow a much more uniform distribution. Unfortunately, however, the observational situation for dwarfs themselves does not support this theory<sup>10</sup>, so either we have a dubious understanding of the formation mechanism for dwarf galaxies, or the biasing mechanism itself does not play a significant part in the formation of large-scale structure.

But Crotts's results are tentative. To begin with, the quasar spectra used in the analysis have abrupt changes in the signal-to-noise ratio along the spectrum, so it is difficult to identify absorption lines uniformly. In fact, two of the four quasars had previously been studied<sup>11</sup>. A comparison of the absorption line lists common to both studies reveals several differences. This previous study<sup>11</sup> did not find any evidence for a positive cross-correlation signal. Also, the technique Crotts uses to identify blended absorption features in his data is different from that generally used; it is important to be confident that over

interpretation of the data does not result in enhanced apparent clustering (particularly for the stronger absorption lines, for which Crotts finds the most significant correlations).

Our recent search for any tendency for clustering amplitude to depend on absorption line strength along single sightlines was negative<sup>7</sup>, indicating that if any effect exists, it has to be only on very small scales, where the data are least reliable. Nevertheless, the general tendency Crotts has found for line positions to cross-correlate is convincing, and if correct demonstrates that more surveys should produce exciting results and perhaps enable us to map the clustering of matter at different epochs.

Another important issue which Crotts discusses is the possible presence of large-scale features in the distribution of Lyman- $\alpha$  clouds. The controversy raised by Crotts is whether or not there exist voids in the Lyman- $\alpha$  forest, analogous to those seen in the galaxy distribution at lower redshift<sup>12</sup>. In an earlier paper<sup>13</sup> he presented evidence for a statistically significant gap in the spectrum<sup>14</sup> of the high-redshift quasar 0420-388 (Fig. 2). This claim was preceded by an analysis of the same data by Carswell and Rees<sup>15</sup> who found no such features. Following on from this, Ostriker *et al.*<sup>16</sup> claimed that Crotts's gap is statistically insignificant, a point which Crotts now rejects<sup>1</sup>. The main point of contention concerns the method of estimating the *a priori* probability of finding a 46 Å region of spectrum at  $z = 2.58$  devoid of Lyman- $\alpha$  absorption lines. Complications arise because the probability of finding any gap is a function of the true number density of absorption lines. Because of absorption-line blending, the number we actually see is lower than the true number.

Whether or not Crotts's gap is a real void, a larger body of data is needed to resolve the issue. About 20 quasar spectra which can be used for a void search are known. In Fig. 3, I compare a simple analysis of the maximum intervals observed

in 22 spectral regions with an estimate of the distribution expected for a randomly distributed population of Lyman- $\alpha$  clouds (derived empirically from a large set of numerically simulated quasar spectra; details of both the data used and the numerical simulations can be found in ref. 7). Clearly, the agreement between the two curves is remarkable, and there seems to be no evidence for any void structure. This being the case, it appears that if the QSO0420-388 void is real, it is a rare event, and not indicative of the general Lyman- $\alpha$  cloud distribution. A point worth noting is that the 46-Å gap under discussion actually appears to form part of a much larger region, 4,320-4,440 Å, where the average absorption-line strength seems rather low compared to the mean. One possibility that could be looked into is that there exists an as yet undiscovered quasar at  $z = 2.6$  close to the line of sight to QSO0420-388, causing an increase in the ionizing radiation levels, and consequently a reduction in the neutral-hydrogen fraction for the neighbouring Lyman- $\alpha$  clouds. However, for a gap this large, the inferred comoving distance is enormous and a very close-by object would be required to inflict such damage.

A difficulty inherent in all this work is the availability of suitable quasars — the objects must be bright enough to be able to project reasonably high-resolution spectra, and of course must lie close enough together in the sky and in redshift to be able to sample physically interesting scales. The work discussed here has indicated the enormous benefit of using pairs of groups of quasars as probes of the high-redshift Universe, of which very few are currently known. This highlights the need for continued systematic searches for quasars. It also points to the need for new-generation telescopes with larger collecting areas to enable us to work to fainter limits, where close quasar pairs are more abundant. □

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# Modulating bacterial virulence

Jon R. Saunders

BACTERIAL pathogens frequently exhibit marked variation in their surface components, a phenomenon that assists the sequential colonization of tissues during the course of infections and the avoidance of host immune responses. The underlying molecular mechanisms that drive phase variation, in which expression of a particular component(s) is reversibly switched off and on, and antigenic variation, in which the qualitative nature of the component is altered, vary considerably in different bacteria. It is, however, becoming clear that complex DNA transactions can be responsible for such changes. Uniquely, in the case of gonococcal pilus variation, a gene-transfer event is involved, as demonstrated recently by Siefert *et al.*<sup>1</sup> and, in a paper on page 655 of this issue<sup>2</sup>, by Gibbs *et al.*

The production of filamentous protein adhesins called pili (fimbriae) by *Neisseria gonorrhoeae* (causing gonorrhoea) and its close relative *N. meningitidis* (causing meningococcal meningitis) is subject to a phase variation in which the bacteria can switch reversibly between a piliated ( $P^+$ ) and a non-piliated ( $P^-$ ) state. This is probably advantageous to the bacteria because it allows sequential rounds of absorption and desorption from host mucosal surfaces. At the same time, these bacteria can alter the nature of the pilin polypeptide subunit that makes up pili to effect pilus antigenic variation.

Both forms of variation arise as a consequence of recombination events in which variant pilin sequences from one of several silent (*pilS*) loci are transferred to a single pilin expression (*pilE*) locus<sup>3,4</sup>. Depending on the precise nature of these events, the end product is a *pilE* locus capable of expressing either functional and possibly antigenically variant pilin, or one of several 'unorthodox' pilins that alter the piliation phenotype, usually to generate a  $P^+ \rightarrow P^-$  phase transition<sup>4,5</sup>. Gonococcal S-pilin variants, for example, produce a soluble pilin that is excreted and results in a reduced level of pilus production, whereas L-variants produce over-length pilins that are not assembled into mature pili. Such types of  $P^-$  variant are produced by a reversible process that, like pilin variation itself, is *recA*-dependent<sup>6</sup>. Reversible  $P^-$  variants that make missense or nonsense pilins can also arise by a *recA*-independent process of base substitution or deletion causing frame shifts<sup>6</sup>. Furthermore, non-reverting ( $P_n^-$ ) variants can be generated by deletion of the 5' end of *pilE* (ref. 6).

Pilus variation in *N. gonorrhoeae* is reduced but not completely abolished in the presence of DNase<sup>1,2</sup> and by mutants

that are defective in the DNA uptake stage of transformation<sup>1</sup>. This suggests that the main part of such variation arises as a result of genetic transformation of recipient cells with *Neisseria* DNA carrying *pil* sequences that has been released by lysis of fellow cells (Fig. 1). *N. gonorrhoeae* and *N. meningitidis* are notable amongst bacteria in being both readily autolytic and naturally highly competent for DNA uptake at all stages of growth. Furthermore, the pathogenic *Neisseria* discriminate against the uptake of heterologous DNA, and in the case of *N. gonorrhoeae* the 10-base pair sequence 5'-GCCGTCTGAA-3' is required for

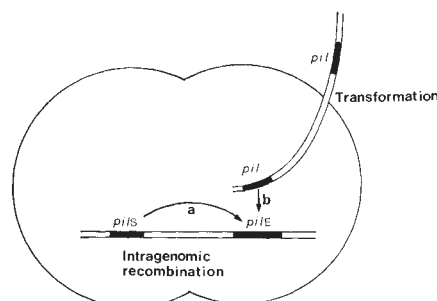


FIG. 1 Two routes for generating pilin variation in *N. gonorrhoeae* by (a) intragenomic recombination and (b) recombination following transformation with exogenous DNA from lysed gonococci: *pilS*, silent pilin sequence; *pilE*, pilin-expression locus complete with promoter; *pil*, any pilin sequence. Note that the incoming donor sequence could be either a second *pilE* locus or any *pilS* locus. Depending on the nature of the *pil* sequence transferred and the outcome of recombination crossovers, the new sequence generated at *pilE* may encode a functional and possibly antigenic variable pilin (antigenic variation) or a non-functional variant pilin which is not assembled into pili to effect one form of  $P^+ \rightarrow P^-$  phase variation. (Subsequent recombination events with further *pil* sequences can reverse the transition.)

efficient uptake of DNA molecules. This uptake sequence is rare in heterologous DNA but occurs frequently in gonococcal DNA, notably in transcription terminator regions<sup>7</sup>. All types of reversible  $P^-$  variants are competent for transformation and could therefore regain the ability to produce pilin by such a route<sup>2</sup>. In contrast,  $P_n^-$  variants cannot be transformed and cannot regain the ability to produce pilin. Because such variants are also avirulent, it is questionable whether  $P_n^-$  bacteria have any significance in the natural pathogenesis of gonorrhoea. The need to generate variation by the acquisition of *pil* sequences may explain the maintenance of an efficient and highly specific system for transformation by gonococci. Intra-specific gene transfer as an essentially

routine mechanism for modulating expression of a crucial gene is, so far, unique to the gonococcus. A proportion of pilin variation events occur in the presence of DNase as a consequence of a second pathway involving intragenomic reciprocal recombination between *pilE* and one of the silent *pilS* regions (Fig. 1). The balance between the two pathways may be dependent *in vivo* on population density which would affect the availability of free DNA for transformation.

Gonococci and meningococci produce a second antigenically variant virulence determinant in the form of heat-modifiable outer membrane proteins (also called opacity proteins or P.II). Unlike pilin expression which is controlled by a single transcribed gene, each P.II polypeptide is encoded by a separate gene (*opa*) of which there are at least nine in *N. gonorrhoeae* and four in *N. meningitidis*<sup>8,9</sup>. A single gonococcal strain may express no P.II or a varying number (up to about six) of the antigenically different polypeptides. Most of the amino-acid sequence of these polypeptides is conserved between P.II species, apart from two short, variable, hydrophilic regions which are located on the outer surface of the outer bacterial membrane. Each protein exhibits independent off  $\leftrightarrow$  on variation at a rate of about  $10^{-3}$  to  $10^{-4}$  per cell per generation.

Although recombination may occur between different *opa* genes, it is now clear that the principal mechanism for effecting the phase and antigenic variation of P.II is *recA*-independent<sup>10</sup>. All the *opa* genes in the cell are transcribed constitutively<sup>8</sup>; modulation of expression is achieved by alteration in the DNA sequence encoding the hydrophobic core of the leader peptide, which acts as a signal sequence and is cleaved off during transport of P.II across the inner membrane. This DNA sequence contains between 7 and 27 direct repeats of 5'-CTCTT-3' (refs 8 and 9). When the number of repeats is 9, 12, 15 and so on, the coding sequence for mature P.II polypeptide is in-frame with respect to the leader peptide, and functional polypeptide is produced after processing (Fig. 2). But when the number of repeats is altered to, say, 13 or 14 the coding sequence will be out of frame, resulting in

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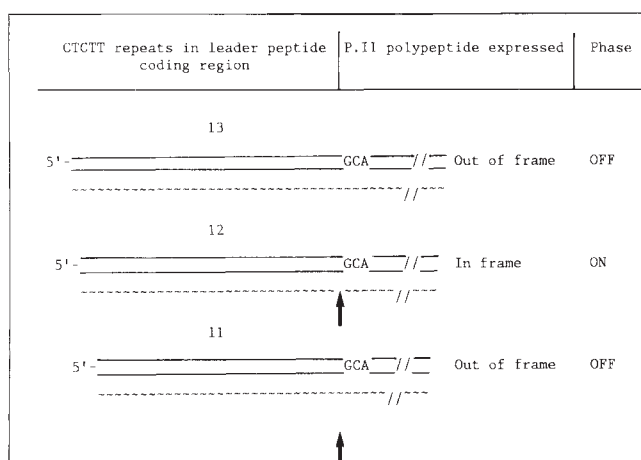


FIG. 2 Slipped-strand mispairing as a mechanism for generating P.II phase and antigenic variation in *N. gonorrhoeae*. Arrow, processing of leader peptide on amino-terminal side of alanine residue.

a nonfunctional polypeptide. The most likely mechanism for producing this phase variation is slipped-strand mispairing, which would occur following local denaturation of DNA and subsequent mispairing of bases within different copies of the tandem CTCTT repeat unit. This process will result in insertion or deletion of unit numbers of repeats<sup>8,10</sup>.

Slipped-strand mispairing has been proposed as a general mechanism for DNA amplification<sup>11</sup> and may account for the creation of hotspots at which frameshift mutations occur both at tandem short sequence repeats<sup>12</sup> and at runs of a single base<sup>13</sup> within a sequence. A similar mechanism for generating frameshifts could also account for the modulation of virulence in *Bordetella pertussis*<sup>14</sup>. The product of the *vir* gene of this bacterium is required for the coordinate expression of various virulence-associated proteins including toxins and haemolysin. Expression is regulated by a spontaneous phase variation ( $\text{Vir}^+ \leftrightarrow \text{Vir}^-$ ) and also by environmental conditions. The *vir* gene product is similar to so-called two-component sensory-regulatory proteins

which respond to environmental stimuli. It is unusual in that it exhibits in one polypeptide both the inner-membrane sensor and the regulator functions normally contained in separate proteins. The crucial event that causes the phase transition from  $\text{Vir}^+$  to  $\text{Vir}^-$  and *vice versa* is a frameshift in the open reading frame for the 'regulator' domain of the *vir* gene product<sup>14</sup>. The insertion of an additional cytosine residue in a run of six cytosines in *vir* converts the bacterium from  $\text{Vir}^+$  to  $\text{Vir}^-$  by disrupting the *vir* open reading. The sequence around the site of the frame shift is notable for its near symmetry and for its high guanosine + cytosine content which could possibly stimulate slipped strand mispairing or some alternative process. It is also noteworthy that frameshift mutations that lead to reversible  $\text{P}^+ \rightarrow \text{P}^-$  phase transitions in *recA*<sup>-</sup> strains of *N. gonorrhoeae* can arise by the insertion or deletion of cytosine residues in a run of eight cytosines located approximately in the middle of the pilin open reading frame<sup>6</sup>.

Bacteria are obviously capable of exploiting mechanisms other than transcriptional control to modulate virulence. What is not yet clear is whether such processes as specific homologous recombination events and slipped-strand mispairing are themselves under some sort of programmed control or operate randomly to generate genetic variation. □

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are several obvious questions: what are the time and temperature dependencies of the various motions? Are they inter- or intramolecular? If intramolecular, which parts of the protein are involved? Are the times more or less uniformly distributed over the entire range or does a hierarchy exist<sup>4</sup>? What is the connection between different motions and the function of a particular protein?

The difficulties involved in answering these questions become apparent when proteins are compared with glasses. In glasses, two types of motions, called  $\alpha$ - and  $\beta$ -relaxations, are well known<sup>5</sup>. Despite many years of work on different glassy systems, no universally accepted theory exists and their properties are not fully explored. Because proteins are far more complex than ordinary glasses, their motions would be expected to display a richer spectrum, less accessible to experiment or theory. Indeed, the theoretical description of motions in proteins is far from complete. The most successful approach, based on molecular-dynamics calculations<sup>6</sup>, can treat motions only up to times of a few hundred picoseconds. A comparison of theory and experiment hence requires experiments that can measure motions between, say, 0.1 and 100 picoseconds.

The papers by Doster *et al.*<sup>2</sup> and by Nienhaus *et al.*<sup>3</sup> partially solve some of the questions listed above and open the way to address some others. In standard scattering experiments with X-rays or neutrons, the intensity of the elastically scattered particles is observed as a function of the momentum  $q$  transferred to the scatterer. Because, loosely speaking, the spatial coordinate  $x$  is conjugate to  $q$ , elastic-scattering experiments can give information about the geometry of the protein motions, but not about their time dependence<sup>7</sup>.

In the inelastic neutron-scattering experiments of Doster *et al.*, the scattered intensity is observed as a function of  $q$  and of the energy transferred to the protein. Because the energy is related to the time, such experiments also provide information about the time dependence of protein dynamics. Doster *et al.* indeed observe two different relaxation processes,  $\alpha$  and  $\beta$ , in the time window between 0.1 and 100 picoseconds. A meaningful comparison with molecular dynamics now becomes possible.

Nienhaus *et al.* use the  $\gamma$ -rays from a Mössbauer source to observe diffuse scattering from a myoglobin crystal. They separate elastic and inelastic scattering and show that the motions in myoglobin at temperatures above 200 kelvin are predominantly intramolecular.

Although both these experiments answer some of the questions I mentioned above, they also raise a host of new ones. One of the most intriguing of these

## MOLECULAR DYNAMICS

# New looks at protein motions

Hans Frauenfelder

It is now generally accepted that protein motions are crucial for the function of biological macromolecules — see Peter Artymuk's News and Views article<sup>1</sup> last year, for example. The goal of characterizing and classifying all motions, however, is still distant. Present experimental techniques permit only studies of some types of motions, and theory and experiment can be compared meaningfully only in a few instances. Two new papers report progress in obtaining the desired experimental information: Doster, Cusack and Petry<sup>2</sup> use inelastic neutron scattering to

explore fast motions in myoglobin over the temperature range from 4 to 350 kelvin; whereas Nienhaus *et al.* on page 669 of this issue<sup>3</sup> measure the molecular dynamics of a myoglobin crystal with the sharp X-rays from a Mössbauer source.

The characteristic times of motions within proteins at physiological temperatures range over more than 12 orders of magnitude, from below picoseconds to well above seconds. Motions can occur as equilibrium fluctuations or as nonequilibrium processes, induced for instance by a light pulse or a biological reaction. There



new problems is based on the observation of Doster *et al.* that their  $\beta$ -motions can be described with a simple two-well model, with parameters similar to those found in earlier very different experiments<sup>8</sup>. This simplicity is reminiscent of the situation in amorphous solids where different glassy systems all exhibit similar low-temperature properties, 'explained' by the two-level states. The apparent simplicity suggests a deeper phenomenon, both in protein motions and in glasses<sup>9</sup>, that unifies the dynamics.

A critical look at progress in scattering and diffraction techniques shows that the elegant experiments of Doster *et al.* and of Nienhaus *et al.* are not the end of the story, but the start of a promising era. Progress in X-ray and neutron sources, the development of better detectors and more powerful computers, and the introduction of cryogenic and high-pressure

techniques, all indicate that progress in protein dynamics will be rapid. It could even be that theory and experiment will be successfully combined so that the relation between dynamics and function will finally be understood.  $\square$

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## FLUID DYNAMICS

# Spreading it about

Philip Ball

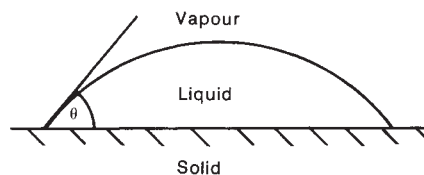
A LIQUID droplet deposited on a solid surface can do one of three things: spread as thinly as possible, which is to say, to monolayer thickness; form a cap of greater average thickness; or refuse to spread at all. That this choice should depend on such physical properties as the surface roughness and the purity of the liquid, as well as the thermodynamic 'wetting' characteristics of the interfacial system, is to be expected. That the spreading process should involve such subtle structural phenomena as are reported by Heslot *et al.* on page 644 of this issue<sup>1</sup> is a considerably less predictable outcome. The authors follow the evolution in time of tiny liquid droplets spreading on a surface, and find that the profile of the droplet edge is step-like, developing up to four discrete molecular layers during the course of spreading.

Central to the study of droplet spreading is the concept of equilibrium wetting. Despite the early start on the topic made by Young in 1805, only in the past two decades, owing to new realistic microscopic statistical mechanical theories of fluids, has much progress been achieved. Young's equation, which defines the states of partial and complete wetting and thus points implicitly to the existence of a wetting transition between them, is essentially a force-balance relation describing the mechanical equilibrium of a liquid drop, coexisting with its vapour, resting on a solid surface (see figure).

This equilibrium is characterized by the contact angle  $\theta$ , a macroscopically observable quantity. Cases in which the droplet sits on the surface as a cap with a non-zero value of  $\theta$  correspond to

partial wetting ( $\theta > 0$ ). If the droplet spreads to form a flat film ( $\theta = 0$ ), one has complete wetting. The contact angle is temperature-dependent: in 1977 Cahn<sup>2</sup> predicted that a transition from partial to complete wetting should occur at some wetting temperature  $T_w$  below the critical temperature of the fluid.

In microscopic statistical mechanical treatments, however, states of partial and complete wetting correspond to different types of uniform liquid film on the solid surface (which is generally considered inert and structureless). At liquid-vapour coexistence, complete wetting is still characterized by a macroscopic (an infinitely thick) liquid film, but partial wetting corresponds to a uniform film of finite thickness (typically a few molecular



A liquid droplet, in equilibrium with its vapour, on a solid surface. For mechanical stability, the surface tensions must satisfy Young's equation:  $\gamma_{sv} = \gamma_{sl} + \gamma_{lv}\cos\theta$ , where  $\gamma_{sv}$ ,  $\gamma_{sl}$  and  $\gamma_{lv}$  are the surface tensions of, respectively, the solid/vapour, solid/liquid and liquid/vapour interfaces and  $\theta$  is the contact angle. In thermodynamic terms these surface tensions correspond to the excess free energies, per unit surface area, of the respective two-phase systems owing to the presence of the interface. At the wetting temperature  $T_w$ , the system lowers its free energy by interposing a macroscopic layer of liquid between the solid and vapour; that is,  $\theta = 0$  for  $T \geq T_w$ , so that the droplet will tend to spread.

diameters thick) rather than to macroscopic droplets separated by regions of bare surface. The latter configuration is, in fact, only metastable with respect to this 'thin' film.

For theorists, the wetting transition provides a wealth of interesting phenomena: the transition from a finite to an infinite film may exhibit characteristics of both two- and three-dimensional systems; under different conditions the transition may be first order (discontinuous), continuous, or may exhibit a cross-over between the two; and capillary-wave fluctuations at the edge of a thickening film may be important on the approach to complete wetting. Experimental evidence for a wetting transition for a pure fluid remains sparse; many simple fluids seem to undergo their wetting transition at the bulk triple point (gas-liquid-solid coexistence point). But from the pragmatic viewpoint (for example, lubrication) the pertinent question is not simply that of how wetting occurs, but of how quickly it occurs.

The dynamics at present lacks a complete (or even more than formative) theory, which is why novel observations such as those of Heslot *et al.* in this issue<sup>1</sup> are to be welcomed. One of the most important aspects of spreading dynamics, the existence of the precursor film, is a demonstration of the fact that macroscopic considerations, that is, the existence of a well defined contact angle, do not extend down to the microscopic scale. The precursor film precedes the observable edge of a spreading drop, extending outwards from the central cap to macroscopic dimensions (of the order of a few millimetres) but having a thickness of only molecular proportions (a few ångströms).

Only with the advent of refined microscopic techniques, notably ellipsometry (which measures, with polarized light, the thickness of films down to molecular resolution), has it become possible to examine the structure of this film in any detail. In early studies the film seemed to be a more or less uniform molecular 'carpet'; we now see that its structure can be both complex and dynamic. Although the droplets studied by Heslot *et al.* are of such small volume ( $10^{-4}$  microlitres) that there is no central macroscopic region at all (the central cap has a thickness considerably less than a micrometre), the layered precursor film is still clearly visible.

Layering effects in liquids are well established. In computer simulations, modern microscopic theories<sup>3</sup> and experimental observations<sup>4</sup> of inhomogeneous fluids, the density profile of a liquid close to a solid wall exhibits oscillatory behaviour with a period of about a molecular diameter; this is associated with the packing of molecules into layers under the

influence of short-ranged repulsive interactions. Adsorption experiments have long revealed that these effects may be sufficiently pronounced for abrupt transitions to be observed between films with a successively increasing number of layers. The low temperatures at which most previous experiments have been conducted mean that the thin films studied were probably solid-like, in which case pronounced layering and sharp (first-order-like) transitions come as no surprise. But more recent adsorption experiments, close to and above the triple point of the adsorbate, demonstrate that the transitions can persist in films that are clearly liquid-like<sup>5</sup>.

But these are all equilibrium observations. It is not clear how the appearance of layering away from equilibrium should be interpreted. If the layering seen by Heslot *et al.* bears any relation to the equilibrium phenomena, one could be tempted to suggest the intriguing possibility that under appropriate conditions a droplet may spread not in a smooth, continuous manner but rather in a series of abrupt jumps echoing the equilibrium transitions. On the other hand, the effect may be purely a flow phenomenon; Israelachvili *et al.*<sup>6</sup> observe that the shear stress in thin liquid films confined between two surfaces also shows step-like behaviour resulting from layering effects (the flow rates are, however, very much greater in these experiments).

What is the final state of the spreading drop? It has been thought that, for a non-volatile wetting liquid, the drop becomes a flat 'pancake' with well-defined edges and a thickness of molecular proportions. However, Heslot *et al.*, in related work just reported in *Physical Review Letters*<sup>7</sup>, have found that, for sufficiently long observation times (of the order of weeks), such a stationary terminal state is not achieved. Instead, molecular diffusion occurs at the edges of the pancake, leaving them increasingly ragged as molecules diffuse out over the surface. The droplet profile then comes to resemble a broad gaussian curve, characteristic of diffusion, and the final state is essentially a two-dimensional gas of molecules on the substrate. These observations stress the importance of short-ranged forces in controlling the structure of the precursor film, and the role of molecular dynamics in the final stages of spreading. □

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# Sea-saurians for sceptics

Michael A. Taylor

It is hard to trace the evolution of marine reptiles in the Mesozoic era (250–65 million years ago). The transformation of a terrestrial reptile into a swimming form obscures relationships, and different groups evolve deceptively similar adaptations to the same mechanical problem of life in water. The theory of cladistics provides an objective basis for analysing relationships between organisms<sup>1</sup>. Its value is shown by two important new papers by Sues<sup>2</sup> and by Rieppel<sup>3</sup> attacking the problem of the evolution of sauropterygians. Although Sues and Rieppel differ in some details of interpretation, their work demonstrates the value of cladistics in generating a specific, testable hypothesis of a major adaptive transition from fully terrestrial to fully marine reptiles. Museums are rich in specimens waiting to provide the answers to some of the new questions raised by Sues and by Rieppel.

The order Sauropterygia is conventionally thought to comprise two groups of predatory marine reptiles, the Nothosauria and the Plesiosauria. The nothosaurs of the Triassic (250–210 million years ago) swam by beating their tails from side to side, and some also used their limbs in a rowing action. By contrast, the plesiosaurs of the later Mesozoic had short, rigid bodies, weak tails and four wing-like hydrofoil limbs with which they 'flew' through the water<sup>4,5</sup>. Nothosaurs could walk or crawl on land, but plesiosaurs may not have been able to move on land.

Despite these differences, overall similarities suggested that the nothosaurs were the ancestors or at least the closest known relatives of the plesiosaurs. But this traditional view lacked cladistic rigour<sup>1</sup>, and a sceptic could equally well argue for independent evolution from a terrestrial ancestor.

Sues's reassessment<sup>2</sup> of the enigmatic sauropterygian *Pistosaurus*, from the Middle Triassic of southern Germany, confirms that it shows features intermediate between nothosaurs and plesiosaurs. He concludes that *Pistosaurus* is the closest relative of the plesiosaurs and that the two groups share a common ancestor within the nothosaurs. The Nothosauria is therefore a paraphyletic group of various primitive sauropterygians, forcing the abandonment of its use as a formal taxonomic grouping. The concept of 'nothosaur' is not even useful as a grade of adaptation, as Sues documents a transition from the close-inshore and lagoonal adaptation of primitive sauropterygians to the fully marine plesiosaurs (Fig. 1). The undulatory swimming with body and tail of the amphibious primitive forms gives a spectrum through the tail- and limb-

mediated propulsion of more advanced forms to the true limb-mediated propulsion of *Pistosaurus* and plesiosaurs. Finally, plesiosaurs attained a form of underwater flight rather than pure rowing. This is consistent with palaeoenvironmental data, and the greater energetic efficiency of underwater flight over rowing.

This model immediately suggests questions, for example concerning biomechanics. One apparent problem would have been the evolution of the vertical

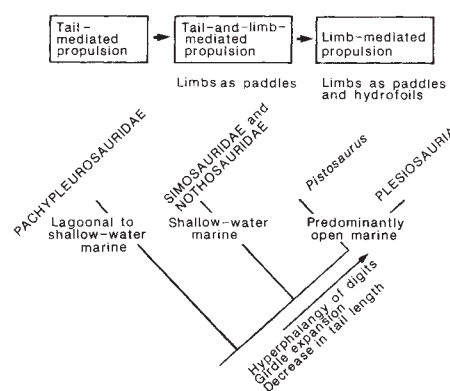


FIG. 1 Evolution of sauropterygian swimming adaptations and palaeoecology, superimposed on a cladogram of relationships of the main groups (from ref. 2).

beat of plesiosaurian wings from the back-and-forth rowing of primitive sauropterygians, but this potential anomaly has already been resolved by the demonstration of a strong backwards component to the power stroke of the plesiosaurian limb action, which thus combines pure hydrofoil action with rowing<sup>4</sup>. Propulsion by limbs rather than a tail fin may confer greater manoeuvrability, as suggested by sea lions compared with sharks. Slow swimming is also hinted by the use of dense ballast (bone in some sauropterygians, stomachs filled with pebbles in plesiosaurs), rather than hydrodynamic forces, to neutralize buoyant lungs<sup>5</sup>, although this explanation is in itself too simple given the many factors controlling an animal's buoyancy.

The underwater flight of plesiosaurs suggests a simultaneous beat of left and right limbs, whereas in swimming terrestrial reptiles and presumably in the primitive sauropterygians the limbs move alternately. An intermediate stage, where the animal used simultaneous or alternate movement as needed, has been suggested.

Rieppel's study<sup>3</sup> of a primitive sauropterygian, the newly discovered pachypleurosaurian *Serpianosaurus* from the Triassic of Monte San Giorgio, Switzerland, shows that this long-tailed form was not a particularly good swimmer or diver, which agrees with Sues's conclusions.

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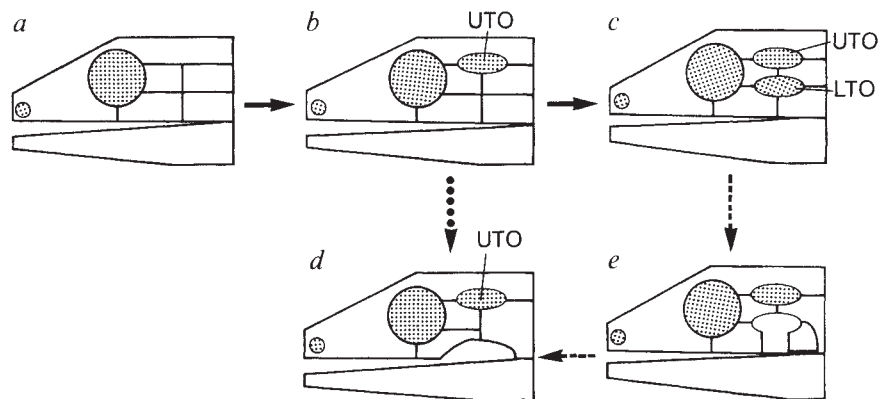


FIG. 2 The sauropterygian skull *d* could have evolved from the primitive reptilian condition *a* through the primitive Diapsida *b* with only the upper temporal opening (dotted arrow: ref. 3). It could also be derived from advanced Diapsida *c* with two temporal openings, by means of the loss of the bar beneath the lower opening (ref. 2) as in the hypothetical intermediate *e* (dashed arrow). LTO, lower temporal opening; UTO, upper temporal opening.

Rieppel's work suggests how the sauropterygian feeding apparatus could have evolved. Terrestrial reptiles manipulate and crush their prey off the ground, and their jaw muscles exert greatest force when the jaws are nearly or wholly closed: a 'static pressure system'. Aquatic tetrapods were thought to have a 'kinetic inertial system' exerting greatest force on the opened jaws, accelerating the lower jaw so as to snap onto prey. Rieppel argues that this dichotomy is too simple. *Serpianosaurus* had the 'static pressure' system, as befits an animal presumably not far removed from its terrestrial ancestry. But the more advanced sauropterygian *Simosaurus* combines both systems in its jaw musculature: an anterior portion exerting maximum force at wide gape, and a posterior portion exerting maximum force near closure. Rieppel asserts that the functional significance of this dual pattern is uncertain, but he may be unduly pessimistic. The dual pattern occurs in at least one plesiosaur, suggesting the possibility that advanced sauropterygians were adapted to predatory feeding, closing the jaw quickly and clamping it shut to kill prey, then if necessary shaking or twisting the prey to pieces<sup>7</sup>.

The origin of the Sauropterygia is another old problem rejuvenated by the use of cladistics. The temporal openings in the cheek of the reptilian skull, behind the eye, provide locations for the tendons and membranes which anchor the jaw musculature. There are two possible locations between the bones of each side, giving upper and lower openings. Reptiles evolved various patterns of these openings, which did not exist in primitive reptiles. Sauropterygians had an 'euryapsid' skull with only the upper opening. But the dominant group of reptiles, the Diapsida, had or have a skull with both upper and lower openings, except the most primitive forms which rather confusingly only had the upper opening. Sues<sup>2</sup> and Rieppel<sup>3</sup> agree that sauropterygians are members of the Diapsida although not

on their precise position within the group (Fig. 2). Sues implies that the apparently euryapsid skull of sauropterygians is secondarily derived from a true diapsid skull through the loss of the bar under the lower opening, as he groups the sauropterygians as a sister group of the Lepidosauromorpha (lizards, snakes, tuataras and their allies). But Rieppel considers that sauropterygians are primitive Diapsida which came off the family tree before the more advanced Diapsida evolved the lower opening, and therefore before the split between the major groups of true diapsids, the Lepidosauromorpha and the Archosauromorpha (crocodiles, dinosaurs, birds, pterosaurs and their allies).

Sues and Rieppel also disagree on the relationships of the Triassic placodonts, shallow-water marine reptiles several metres long, with walking limbs and swimming tails. These reptiles ate hard-shelled invertebrates on the sea bottom, so had to dive to obtain their prey and then crush them with the massive, pavement-like teeth on their jaws and palates. The placodont skull is euryapsid and the placodonts have often been classed with the sauropterygians in the Euryapsida. Sues argues<sup>4</sup> that placodonts are not closely related to sauropterygians. They are, however, advanced Diapsida which lost the lower opening when the cheek bars became massively thickened to resist the stresses of shell-cracking. Rieppel restores the placodonts to a close relationship with the sauropterygians, effectively reinstating the Euryapsida as a monophyletic group within the advanced Diapsida. □

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## Red-hot rhythm

A MIXTURE of gas and oxygen is only explosive between certain limits. Too much gas, too much oxygen, or too much inert gas in the mixture, and flame will not propagate. To complicate things further, these explosion limits vary with pressure.

And this is where Daedalus gets interested. Since explosion itself raises the pressure, a mixture of this sort could in principle form an amplifier. DREADCO's chemists are therefore filling a long hot tube with various cunning near-the-limit gas mixtures, and launching sound down the tube from a loudspeaker. In the sonic pressure peaks the mixture will burn faster and hotter, increasing their pressure still more. Conversely, the flame will be damped or even extinguished in the low-pressure troughs, lowering their relative pressure further. So the sound should be amplified in its journey down the pipe. Provided the mixture is just at the edge of its explosion limit in the hot tube, it will not have time to burn completely during the brief passage of any one pressure peak. Plenty of unburnt gas will be left behind for the next one. But for true continuous operation, top-up gas must be steadily leaked into the tube from many tiny apertures all down its length.

This elegant sound amplifier has no moving parts and only molecular inertia, and can easily handle hundreds of kilowatts of combustion energy; so when perfected it should be the ultimate in deafening high fidelity. A sound whispered into one end of the tube should emerge in thunderous intensity and perfection from the other. The whole thing is a sort of sonic travelling-wave-tube amplifier pumped by the copious energy of combustion. Bypassing electronics entirely, but running hotter than any valve amplifier, it will be ideal for really large-scale public address, sonic condensation of rain clouds, bouncing sound off the ozone layer, and other appealingly macho technological exploits.

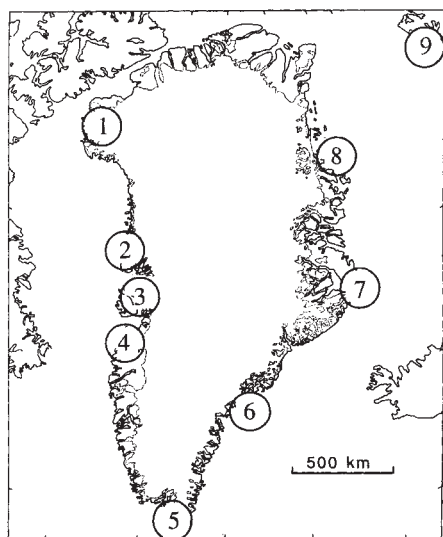
It will, however, need the most scrupulous exponential-horn output termination, giving it the appearance of a huge red-hot trumpet. For a simple square-cut termination would partially reflect the sound output back into the tube. And if only a little of the output gets back to the input in this manner, the result will be a sonic laser, beaming out a hideous and deafening oscillation. While the pop scene might welcome this ultimate in high-output wind instruments, Daedalus plans to silence and harness the monster by terminating its tube in a piston and crankshaft. The speed of the resulting simple sonic motor could best be controlled by varying the length of the tube in trombone fashion. Unburdened by valves or distributor or camshaft, it might seriously challenge the internal combustion engine.

David Jones

# Clue to seal epizootic?

SIR—By testing for the presence of antibodies to canine distemper virus (CDV) in blood samples collected from seals in Greenland during 1984–87, we have obtained evidence for the presence of a morbillivirus related to CDV in Arctic seals before the epizootic that killed more than 17,000 seals in Northern Europe<sup>1</sup> in 1988 and several thousand in Lake Baikal<sup>2,3</sup> in 1987–88.

Of blood (and tissue) samples collected from about 1,600 Greenland seals as part of a project to measure heavy metal



Location of sampling sites.

concentrations in the Greenland marine environment, we have tested samples from ten ringed seals (*Phoca hispida*) from each of nine localities in Greenland and Svalbard (see figure) and from ten harp seals (*Pagophilus groenlandicus*) from four of these sites. Each group of ten animals comprised three yearlings, three subadults and four adults. Only four of the 90 ringed seals tested positive (two weakly positive) but there were twelve positive tests among the 40 harp seals (see table). Immunoglobulin G prepared from a whole blood sample from a harp seal by ammonium sulphate precipitation and

examined for neutralizing antibodies using the Onderstepoort strain of CDV, had a neutralizing titre of 1/256, confirming<sup>4,5</sup> that these antibodies were induced by a morbillivirus closely related to CDV. The year of sampling does not explain the differences between the two species tested.

We conclude that CDV or a closely related morbillivirus was present in harp seals and ringed bells before a similar virus first appeared in seals in northern Europe or Lake Baikal. It is not clear whether, and if so how, the virus spread from the Greenland seals to Europe, or what relationship, if any, there is between our observations and the outbreak of canine distemper in sledge dogs in north-west Greenland during the winter of 1987–88. It has been suggested<sup>7</sup> that the large-scale migration of harp seals from the Barents Sea to north Europe in 1986–87 was responsible for the spread of the viral disease, but Barents Sea harp seals have not yet been tested for the presence of CDV antibodies and this pathway could hardly explain the infection of the Lake Baikal seals.

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## What's in a name?

SIR—Newman<sup>1</sup>, in answer to Crisp and Fogg's letter<sup>2</sup> concerning changes in systematic names, argues that a reversion to the use of subgenera is unsatisfactory. We are in entire agreement with this view. If the evidence of taxonomists dictates the transfer of a species to another genus, or creating a new genus, so be it. "Messing up past literature" and "imposing additional burdens on the memory" are insufficient reasons to warrant an overhaul of the taxonomic system<sup>3</sup>.

The solution is not to alter the behaviour of taxonomists but to address and find solutions to the difficulties that progress in taxonomy causes to the scientific com-

munity. A mechanism is needed to express taxonomy in a way that avoids the confusion and discontinuity in the literature which results from changes in assignment and conflicting opinions.

We suggest that when the generic assignment of a species has been changed, an interchangeable trinomial of the form genus, (=genus), species is used. For example, the barnacle species that is referred to in current literature as both *Semibalanus balanoides*<sup>3</sup> and *Balanus balanoides*, would be named *Semibalanus* (= *Balanus*) *balanoides* (or vice versa). The trinomial would avoid confusion in the literature, allow understandable sentimental attachment to a name, and eliminate the requirement that authors know the prior history of the name in order to trace information in the literature.

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## Bird population densities

SIR—Pimm and Redfearn<sup>1</sup> claim to have shown that, in a sample of 100 populations of animals, there is a general tendency for the between-year variance in population density to increase as one considers longer spans of years. They reasonably conclude that most populations show long-term changes in size, in addition to fluctuations between successive years, and suggest that these patterns may be related to similar patterns in physical variables.

Unfortunately, 74 of the populations considered are of birds counted in the common bird census (CBC) of the British Trust for Ornithology, in which long-term trends may appear merely as artefacts. In the CBC, sample plots are censused annually. Because there is an annual turnover of about one plot in eight, the population is indexed by using plots counted in two successive years to give a measure of population change between those years and then stringing together successive changes to give an index of population numbers against a base year. Thus, the index in one year is correlated with that in previous years and, because the measure of population change between successive years is subject to sampling error, the population index may drift away from the base value even if the population numbers themselves do not change.

The likely magnitude of this random drift has been considered previously. Although it is probably less in the CBC than in some similar censuses<sup>2,3</sup>, it can give

| No. of seals with antibodies (year) |                 |            |
|-------------------------------------|-----------------|------------|
| Locality<br>(no. on map)            | Ringed<br>seals | Harp seals |
| Thule (1)                           | 2 (1985)        | 1 (1985)   |
| Upernavik (2)                       | 0 (1985)        | 6 (1985)   |
| Umanak (3)                          | 0 (1987)        | 4 (1985)   |
| Kangatsiaq (4)                      | 0 (1986)        | 1 (1986)   |
| Nanortalik (5)                      | 0 (1986)        |            |
| Angmagsalik (6)                     | 0 (1987)        |            |
| Scoresbysund (7)*                   | 2 (1986)        |            |
| Danmarkshavn (8)                    | 0 (1984/86)     |            |
| Svalbard (9)                        | 0 (1986)        |            |

CDV-antibody detection by an indirect fluorescent antibody test with FITC-conjugated protein A.

\*Weak response



the appearance of genuine long-term trends. Furthermore, although there is a good correlation between the CBC index and estimated true densities<sup>2,4</sup> (suggesting that only a small part of the variance in the index values results from sampling error), in some species correlation between the index and estimated true densities is apparently weak. The important question is whether random variation of reasonable magnitudes could produce an increase in variance of the magnitude demonstrated by Pimm and Redfearn. I have carried out simple simulations that suggest that it could.

Because there is good evidence for systematic changes in the populations of British birds<sup>4</sup>, I do not believe that Pimm and Redfearn's conclusions should be dismissed, but merely that they are less well-founded than appeared at first sight.

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SIR—I suggest that there is an alternative explanation for Pimm and Redfearn's<sup>1</sup> observations that is consistent with a white-noise hypothesis of environmental variation in terrestrial environments<sup>5</sup>—at least over the timescales they examined (up to 30 years).

The phenomenon, the standard deviation of the logarithm of the annual density (SDL) increasing with the census period, may result from autocorrelation between densities in adjacent years; they will be more similar than years that are far apart. In the redshift model the populations are being driven by an autocorrelated environment. An alternative explanation is that the observed autocorrelation results from the population dynamics of the animals themselves.

The density in year  $t$  may be similar to that in  $t-1$  because the same animals may be present at both times, or because it depends on the number of breeders available in the previous year. The environmental influences can then be uncorrelated white noise, and the same pattern of SDL increasing with census length will appear. The autocorrelation is thus present despite the environment, not because of it. Such a model, density depending only on the number in the previous year plus a random environmental perturbation, is called a first-order autoregressive (AR1), or Markov, process<sup>6</sup>. Such autoregressive models can give redshifted spectra<sup>7</sup> for the populations' series even though the environmental driving variables are white noise. Indeed, any relatively smooth time series will have its power concentrated at the lower end of the spectrum<sup>8</sup>.

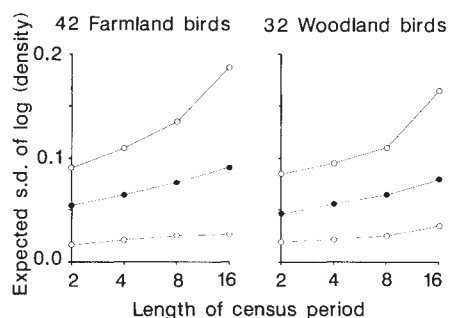
If the data do follow an AR1 model, then the expected value of the SDL from a short census period will underestimate the

true variability of the population. But as the census period increases, the expected value of the sample SDL will asymptotically approach the true value. Thus Pimm and Redfearn's effect could be the result of small sample bias in the estimator of the true, constant, SDL. The expected value of the SDL,  $(E[s])$  is given by,

$$(E[s])^2 = \frac{N}{N-1} \sigma^2 \left\{ 1 - \frac{1}{N} - \frac{2}{N} \left[ \sum_{r=1}^{N-1} \left( 1 - \frac{r}{N} \right) \rho^r \right] \right\}$$

(ref. 9) where  $\sigma$  is the true SDL,  $\rho$  the autocorrelation and  $N$  the number of years in the census.

To determine whether this small sample bias could explain Pimm and Redfearn's results, the autocorrelations were calculated for the farm and woodland bird populations used in their paper, and then corrected for their own small sample bias<sup>9</sup>. Though the data sequence is too short for



Expected SDL if the populations follow an AR1 model, calculated using the equation given, against the length of the census period. The mean, maximum and minimum SDLs calculated over the species are plotted at each census length.

formal investigation, the partial autocorrelation structure of most of the species was consistent with an AR1 model, though in some species there was support for a higher-order model. There was evidence for long-term trends (non-stationarity) in only six of the 74 populations studied. Using the SDLs for the 16 years as a starting point, the SDLs for 2-, 4- and 8-year censuses were calculated for each species using the above equation. The mean, maximum and minimum for each census length are displayed in the figure, they are similar in shape and magnitude to those of Pimm and Redfearn. Had higher-order models been fitted, the curves would be even steeper, but the quality of the data does not justify a more complex model.

Thus, the available data do not allow us to distinguish between the white and red-noise hypotheses of environmental variation.

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PIMM AND REDFEARN REPLY—Not surprisingly, there are many explanations for the increase in temporal population variability over progressively longer time spans<sup>1</sup>. These explanations are not mutually exclusive, and no explanation has logical priority. Whatever the explanations, the implications for community dynamics remain.

Part of the increase, for some species, can be explained by certain sampling problems, but this cannot apply to all the data in which increases are found. A population-level explanation comes from the consequences of a population's dependence on the densities of previous years. Long-lived species or those with density-dependent dynamics will show strong autocorrelations between years.

There are also community-level explanations. There is abundant evidence that each species' dynamics depends on many other species in the community. Simple, multi-species population models can produce chaotic dynamics with redshifted spectra<sup>10</sup>. An ecosystem-level explanation is that abiotic variables determine densities both directly and indirectly by altering species' access to resources and their escape from enemies. Clark's<sup>11</sup> investigation of the frequencies of fires is just one independent demonstration of how constantly changing physical processes drive terrestrial populations.

A single explanation for the increase in variability is unlikely. It is found in populations with complex cyclical changes in density, probably caused by interspecific interactions. The increase is also found in populations of long-lived birds and mammals (which have high year-to-year autocorrelations). But there is also more variability over 32 years than 16 years in a newly analysed set of seven moth<sup>12</sup> and three diatom species<sup>13</sup>. These species are short-lived; for insects demonstrations of density-dependent processes are few.

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# Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene

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Maternal *hunchback* activity suppresses the genetic pathway for abdomen formation in the *Drosophila* embryo. The active component of the posterior group of maternal genes, *nanos*, acts as a specific repressor of *hunchback* in the posterior region. Absence of both repressors results in normal embryos, indicating that posterior segmentation may not directly require maternal determinants.

SEGMENTATION in *Drosophila* is controlled by the activity of maternal organizer gene products which are initially localized in the pole regions of the egg<sup>1</sup>. The segment pattern in the anterior region is apparently organized by a morphogenetic gradient formed by the *bicoid* (*bcd*) gene product<sup>2-4</sup>. The first level of zygotic segmentation genes, the gap genes<sup>5</sup>, are assumed to be regionally activated or repressed in direct response to the local concentration of the *bcd* protein<sup>4</sup> in the anterior region. The final segment pattern is then further established by a cascade of interactions among the zygotic segmentation genes (reviewed in refs 6 and 7). It seemed possible that a similar mechanism could apply for the organization of the posterior region<sup>1</sup>. This region is controlled by the posterior group of maternal genes<sup>1,8-10</sup>, which are thought to be mainly involved in the synthesis, localization and translocation of the *nanos* (*nos*) gene product, the active component of the posterior system<sup>1,11,12</sup>. Mutations of *nos* (or of the other genes of the posterior group) prevent abdominal segmentation<sup>1,9-11</sup>. In such mutant embryos the maternal product of the gap gene *hunchback* (*hb*) persists in the posterior half of the early embryo<sup>13</sup>. This correlation suggests that the maternal *hb* gene product is involved in the abdominal segmentation process. But genetic elimination of the maternal *hb* gene product has been shown to have no apparent developmental consequences<sup>14</sup>, suggesting that the maternal *hb* gene product functions as a repressor rather than as an activator. Here we show that maternal *hb* activity can act directly as a suppressor of abdominal segmentation and that the active component of the posterior system, *nos*, acts as a region-specific repressor of maternal *hb* activity. In the absence of both components, the embryos develop a normal abdomen. This demonstrates that the main function of the posterior system is to control maternal *hb* activity, rather than to provide a morphogen for the spatial organization of the abdomen.

## Maternal interference

The gap gene *hb* is expressed both maternally and zygotically<sup>13-17</sup>. The maternally derived *hb* messenger RNA is evenly distributed in the freshly laid egg. But the first translation products from this mRNA which appear during the early

cleavage stages are not evenly distributed. Instead, they form a transient gradient in the posterior half of the egg which decays before the onset of zygotic gene expression<sup>13,15</sup>. This process is controlled by the products of the posterior group of maternal genes. If one of them is mutant, the post-transcriptional control of the *hb* mRNA is abolished and the *hb* protein is distributed throughout the egg during the early stages of development<sup>13</sup>.

We have observed a similar type of protein distribution in

TABLE 1 Phenotypic rescue of embryos maternally mutant for *nos* in the absence of maternal *hb* expression

| Number of host embryos | Adults (males/females) | Fertile females germ-line genotype (number)                      | Phenotype of embryos               |
|------------------------|------------------------|------------------------------------------------------------------|------------------------------------|
| 418                    | 162 (85/77)            | Unknown (3)                                                      | ND                                 |
|                        |                        | TM3/TM3 <sup>a</sup> (1)                                         | wt <sup>a</sup>                    |
|                        |                        | <i>nos</i> ; <i>hb</i> /TM3 <sup>b</sup> (4)                     | wt and zyg. <i>hb</i> <sup>b</sup> |
|                        |                        | <i>nos</i> ; <i>hb</i> / <i>nos</i> ; <i>hb</i> <sup>c</sup> (3) | wt and mat. <i>hb</i> <sup>c</sup> |

Pole cell transplantation experiments show the phenotypic rescue of embryos maternally mutant for *nos* in the absence of maternal *hb* expression. For our experiments we used the *nos*<sup>L7</sup> allele (R. Lehmann, unpublished data) and the *hb*<sup>90</sup> allele<sup>14</sup>. Embryos maternally mutant for the *nos*<sup>L7</sup> allele show complete lack of all abdominal segments<sup>1,11</sup>. To show that this phenotype is germ-line autonomous, we have tested this allele in a pole cell transplantation experiment of the type outlined below. We found that the phenotype is only dependent on the genotype of the germ-line, as shown in Fig. 2c. We noted, however, that the chromosome carrying the *nos*<sup>L7</sup> allele appears to carry a second mutation, which leads to a much lower than expected frequency for the occurrence of homozygous mutant germ-lines in these experiments. The *hb*<sup>90</sup> allele shows the lack of function phenotype of *hb* in homozygous mutant embryos<sup>14</sup>. But, it appears to have a weak neomorphic or antimorphic function, which is particularly evident in pole cell transplantation experiments. Embryos which are maternally mutant for this allele, but zygotically heterozygous, show a high-frequency lack of the second thoracic segment (M.H. and D.T., unpublished data). This feature served as a useful marker in our experiments. Both *nos* and *hb* are located on the third chromosome. A double-mutant chromosome was obtained by a recombination event between the two genes. This chromosome was brought over the TM3 chromosome, which serves as a balancer to prevent further recombination. TM3 is wild-type for both *nos* and *hb*. The pole cell transplantation experiment was essentially as described<sup>1-4</sup>. The donor pole cells came from embryos of a mating of *nos*; *hb*/TM3 heterozygous flies. They were transplanted into embryos, which were maternally mutant for *Ovo*<sup>D1</sup> (ref. 21). Host embryos (418) were injected, of which 39 per cent survived (columns 1 and 2). The females were backcrossed with heterozygous *hb*<sup>90</sup>/TM3 males. Eleven fertile females were recovered, of which eight laid sufficient eggs to analyse between 30–150 embryos of each. The phenotypes were scored in cuticle preparations of first instar larvae before, or shortly after, hatching from the egg shell. a, The cuticle phenotypes of all embryos of this female were wild-type, about half of which survived to adults. The other half died as larvae, as is expected from animals homozygous for TM3. None showed defects in the second thoracic segment. b, The cuticle phenotypes of the embryos of this class of females were either wild-type (3/4), or zygotic *hb* (1/4). About 1/4 of the 'wild-types' did not survive, indicative of homozygosity for TM3. None showed defects in the second thoracic segments. c, The cuticle phenotypes of the embryos of this class of females were either wild-type, or showed the combined maternal and zygotic *hb* phenotype. None of them showed the *nos* phenotype. The 'wild-type' embryos showed lack of the second thoracic segment at a high frequency (~60%). A minor fraction of both classes of embryos showed weak abdominal defects (see Fig. 3). It is as yet unclear, whether this is due to an insufficient rescue, or to a weak residual activity of the *hb*<sup>90</sup> allele. Several of the embryos of each female (20) were allowed to develop to adults. They were individually mated with heterozygous *nos*/TM3 flies. The females of this progeny were then analysed for whether their embryos developed the *nos* phenotype. This was the case for a quarter of them, which is the expected frequency if their parents were all heterozygous for *nos*/TM3. This is the final proof that the mutant *nos* allele was originally homozygously present in the germ-line of the transplanted females.

ND, not determined; wt, wild-type.

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embryos carrying a copy of a *hb*/ $\beta$ -galactosidase fusion gene, that was constructed in the course of analysing the *cis*-regulatory elements of the *hb* gene (Fig. 1) (ref. 17). The construct contains DNA from the *hb* region including the *cis*-regulatory elements and most of the *hb* protein coding region, fused in-frame to the bacterial  $\beta$ -galactosidase ( $\beta$ -gal) gene (Fig. 1a). After germ-line transformation, the zygotic regulation of this construct is largely normal (data not shown). But the maternally derived protein product is not correctly removed from the posterior region (Fig. 1b). This may be due either to an insufficient translational regulation of the hybrid RNA, or to a higher stability of the *hb*/ $\beta$ -gal fusion protein. The transient persistence of this protein in the posterior region of the embryo causes a dominant maternal effect in the abdominal region which is of variable strength. In weak cases only the abdominal segment six is affected, whereas in the strongest cases, the abdominal segments 3–7 are missing (Fig. 1d).

To assess more closely the effects of this ectopic expression on the early fate map of the embryo, we examined the expression pattern of the pair-rule gene *fushi tarazu* (*ftz*). This gene is normally expressed in seven stripes which correspond to the even-numbered parasegments of the embryo<sup>18–20</sup>. In embryos produced by females which carry the *hb*/ $\beta$ -gal fusion gene, the *ftz* expression pattern is affected in the posterior region; that is, stripes 3–6, which give rise to the abdominal segments 2–7, are altered and partially fused (Fig. 1c). The effects on the expression pattern of *ftz*, as well as the defects observed in the

cuticle pattern of late embryos, strongly resemble those observed with the weak (hypomorphic) *oskar* mutant *osk*<sup>301</sup> (refs 9 and 11). None of these effects occur in embryos that received the *hb*/ $\beta$ -gal fusion gene paternally, nor in embryos which showed a homogeneous expression of  $\beta$ -gal protein alone. This proves that it must be the *hb* fusion part of the *hb*/ $\beta$ -gal protein that suppresses the establishment of a normal posterior segment pattern in the abdominal region of the embryo. This suppression must occur before or at the onset of the zygotic gene expression, as can be inferred from the effects on the *ftz* expression pattern.

### Rescue of *nos*

The above experiments show that the maternal *hb* activity can suppress the abdominal segmentation process. It seemed possible therefore that the abdominal phenotypes caused by mutations of the posterior group of maternal genes might be a result of the persistence of the *hb* protein in the posterior region. To test this, we asked whether embryos which are maternally mutant for *nos* could be rescued if at the same time the maternal *hb* expression is genetically removed. We constructed a chromosome which is double mutant for *nos* and *hb*. Embryos which are homozygous for this chromosome develop the lethal zygotic *hb* phenotype and so a homozygous female germ line cannot be established directly. This problem can be overcome by transplantation of germ-line precursor cells ('pole cells') into host embryos<sup>14</sup> carrying the dominant female sterile mutation *Ovo* (ref. 21) (for details see Table 1). For the construction of female

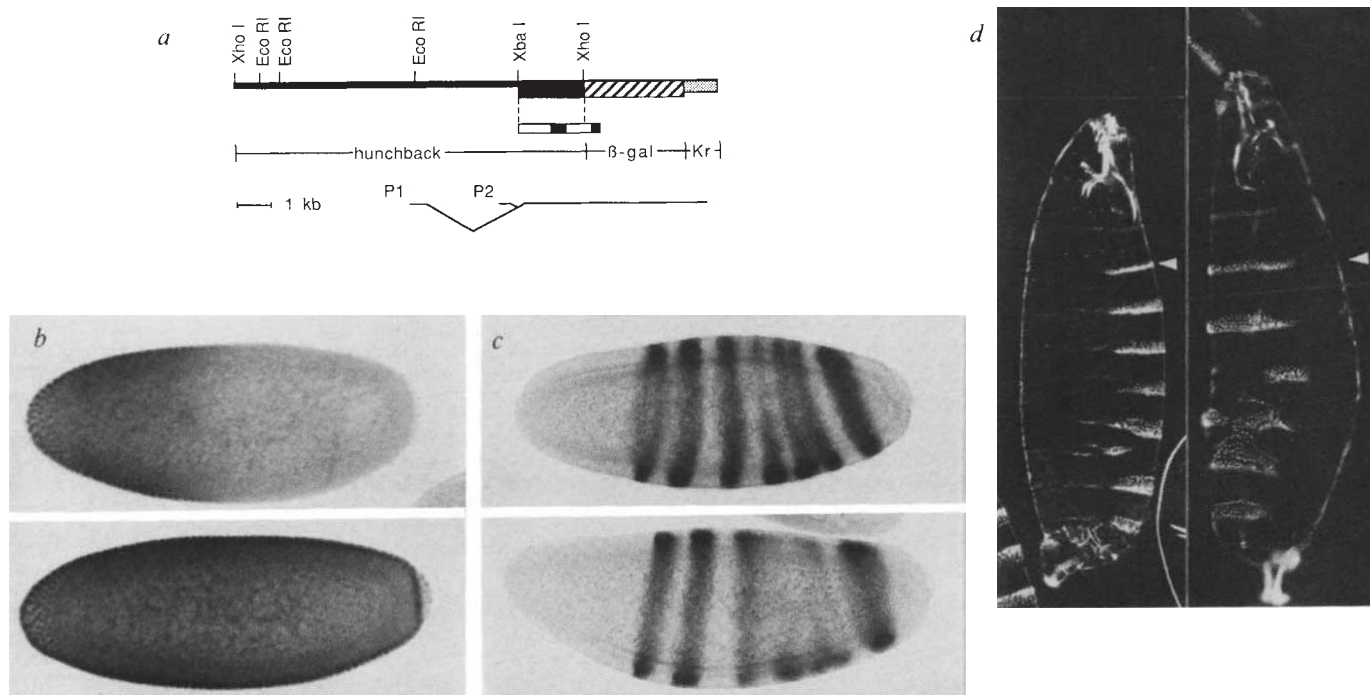


FIG. 1. Interference of the *hb*/ $\beta$ -gal fusion protein with abdominal segmentation. *a*, Structure of the P-element construct. The construct contains a 10.5-kb *Xho*I fragment from the *hb* genomic region<sup>15</sup> (filled box) which was fused in frame to a 3.1-kb fragment from clone pMC1871 (ref. 30) (hatched box), containing the *lacZ* gene of *Escherichia coli*, which codes for  $\beta$ -galactosidase. As polyadenylation signal we used a 0.7-kb *Sau*3A fragment from the *Krüppel* (*Kr*) gene 3' region<sup>31</sup> (stippled box). We found that the use of the *Kr* 3'-trailer region instead of the normally used SV40 3'-trailer region is advantageous for analysing a dynamic expression pattern, because the resultant RNA appears to have a shorter half-life (C.S., unpublished results). The whole construct was cloned in the *Sal*I site of the C20 P-element and used for transformation of *rosy*<sup>−</sup> flies<sup>32</sup>. The box below indicates the normal extent of the *hb* protein with the two finger domains drawn as black boxes. The fusion gene construct contains 583 amino acids out of 758 for the normal *hb* protein. The lines below indicate the presumptive transcripts from the construct with 'P1' being the maternal promoter<sup>15,17</sup>. *b*, Maternal

expression pattern of the normal *hb* protein and of the fusion protein from the *hb*/ $\beta$ -gal construct. The top embryo is a wild-type embryo and is stained with antibodies to the *hb* protein<sup>13</sup>, the lower embryo contains maternally the *hb*/ $\beta$ -gal fusion gene construct and is stained with antibodies against  $\beta$ -gal. Both embryos are about at stage 10–11 (ref. 33), which is shortly before the first zygotic gene expression starts. *c*, The *ftz* staining pattern of embryos containing maternally the *hb*/ $\beta$ -gal fusion gene construct. As with the cuticle pattern, one can observe a range of effects on the *ftz* staining pattern in embryos of these types. The top embryo shows a weak effect, the bottom, one of the most severe effects. Both embryos are at stage 14 (ref. 33). *d*, Cuticle phenotypes of embryos coming from mothers containing the *hb*/ $\beta$ -gal fusion gene construct. The weakest observed effects are on abdominal segment A6 (left), the most severe effects include abdominal segments A3–A7 (right). The first abdominal segment is labelled with an arrowhead in each case. The cuticle pattern of a wild-type embryo is shown in Fig. 2a.

germ lines which are mutant for *hb* and *nos*, we transplanted the pole cells of embryos which were derived from parents heterozygous for *nos*; *hb*. The genotype of the pole cells of these donor embryos could have been homozygous *nos*; *hb*, heterozygous *nos*; *hb*/+ or wild-type. The resulting fertile females will have the corresponding germ-line genotypes, which cannot be directly scored and must be inferred from the range of cuticle patterns of their embryos (for details, see Table 1). The segment pattern of the embryos which derive from a homozygous *nos*; *hb* germ line allows one to see whether the *nos* phenotype can be rescued in the absence of the maternal *hb* gene product. One important prerequisite for this experiment is that the *nos* gene product is germ-line dependent. We therefore showed in a control experiment that a *nos*<sup>-</sup> germ line cannot be rescued by a *nos*<sup>+</sup> soma (see legend to Table 1 for details).

In the experiment with the *nos*; *hb* double mutant chromosome, we recovered eleven fertile females. Three of them carried a germ line homozygous for the *nos*; *hb* double mutant chromosome (Table 1). They were mated to heterozygous *hb* males and the embryos of these matings were examined. None of the embryos which derive from *nos*; *hb* homozygous germ-lines develops the *nos* phenotype. Instead they showed either a normal pattern of segments, or the combined maternal and zygotic *hb* phenotype<sup>14</sup> (*nos*; *hb*/*hb* embryos). This shows that the concomitant removal of the *hb* gene from the maternal germ line fully rescues the *nos* phenotype, regardless of the zygotic genotype at the *hb* locus (Table 1). Some of the embryos which showed a wild-type segment pattern developed into viable and fertile flies. It was thus possible to confirm their genotype by further genetic analysis (see legend Table 1). These experiments with the results above show that the *nos* phenotype is in fact caused by the presence of the maternally derived *hb* protein in the posterior region of the preblastoderm embryo. The same results were obtained using a different allele of *hb*, or using *hb*

in a combination with a different maternal posterior organizer gene, namely *vasa*<sup>22</sup>.

## Discussion

The *nos* gene product is presumably the active component of the posterior group of maternal genes. The other genes in this pathway are believed to generate the *nos* activity at the posterior pole and to transfer it into the prospective abdominal region during the early cleavage stages<sup>1,11,12</sup>. In this context it was reasonable to assume that the *nos* gene product would act as a morphogen rather like *bcd* in the anterior region of the embryo<sup>1</sup>. In the absence of maternal *hb* activity, however, *nos* is dispensable. This observation rules out the possibility that the *nos* gene product acts as a morphogenetic molecule. Instead, *nos* is a direct and region-specific repressor of *hb* at the post-transcriptional level. The proposed activating effect of the posterior group of maternal genes on the posterior gap genes<sup>1</sup> has thus resolved into a double inhibitory effect, namely of *nos* on *hb*, and of *hb* on the zygotic genes required for abdominal segmentation. The most obvious zygotic target gene for repression by the maternal *hb* gene product would be the gap gene *knirps*, the zygotic counterpart of the posterior group of maternal genes<sup>5,11,23</sup>. But the rescue of maternally *nos* mutant embryos also includes the eighth abdominal segment which is not affected in *knirps* mutant embryos<sup>11,23</sup>. This suggests that *knirps* is not the only target gene for the maternal *hb* activity. Further experiments will show whether any of the other gene products of the posterior group of maternal genes has a more specific role in the abdominal segmentation process. The current experiments indicate, however, that the spatial organization of the abdomen may be independent of a morphogen provided by the known members of the posterior group of maternal genes and it is possible that this process could be governed solely at the zygotic level. The establishment of the posterior segment pattern would thus be

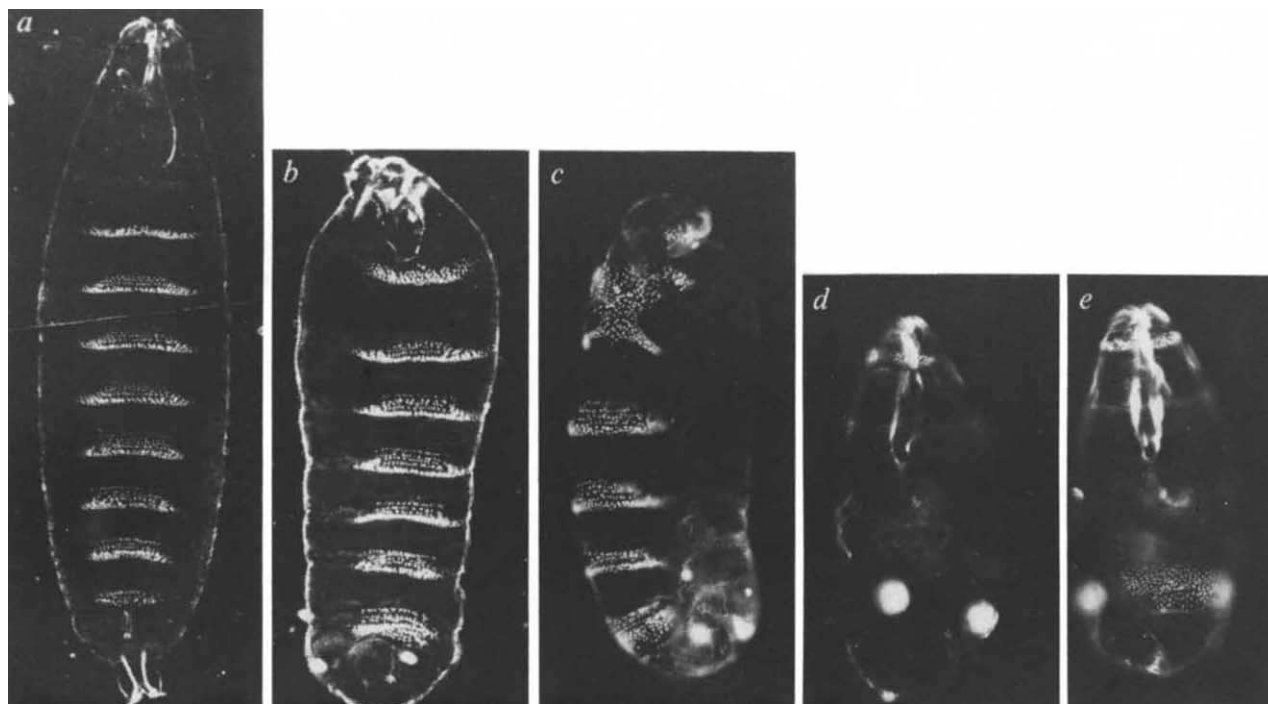


FIG. 2 Cuticular phenotypes of embryos mutant for *hb* and *nos*. a, Wild-type embryo. b, Zygotically *hb* mutant embryo. Labial head segments and thoracic segments T1-3 are missing, abdominal segments A7/A8 are fused<sup>14,25</sup>. c, Embryo showing the combined maternal and zygotic *hb* mutant phenotype. In addition to the zygotic phenotype, abdominal segments A1-3 are partly or fully replaced by a broad, irregularly shaped field of denticles<sup>14</sup>. d, Maternally *nos* mutant embryo. All abdominal segments are missing<sup>1,11</sup>. This embryo was derived from a pole cell transplanted female, whose

germ-line was homozygous mutant for *nos*, but whose soma was *nos*<sup>+</sup> (compare legend to Table 1). e, Embryo from a female, which was a homozygous mutant for *nos* and a heterozygous mutant for *hb*<sup>9Q</sup> (ref. 14). A field of denticles forms in the abdomen, which shows a mirror image symmetry. This effect is temperature-dependent. More denticles belts are formed at 29°C, fewer at 18°C. The phenotype shown develops typically at room temperature. The formation of these denticle belts is apparently due to a partial rescue of the *nos* phenotype in mothers heterozygous for *hb*.



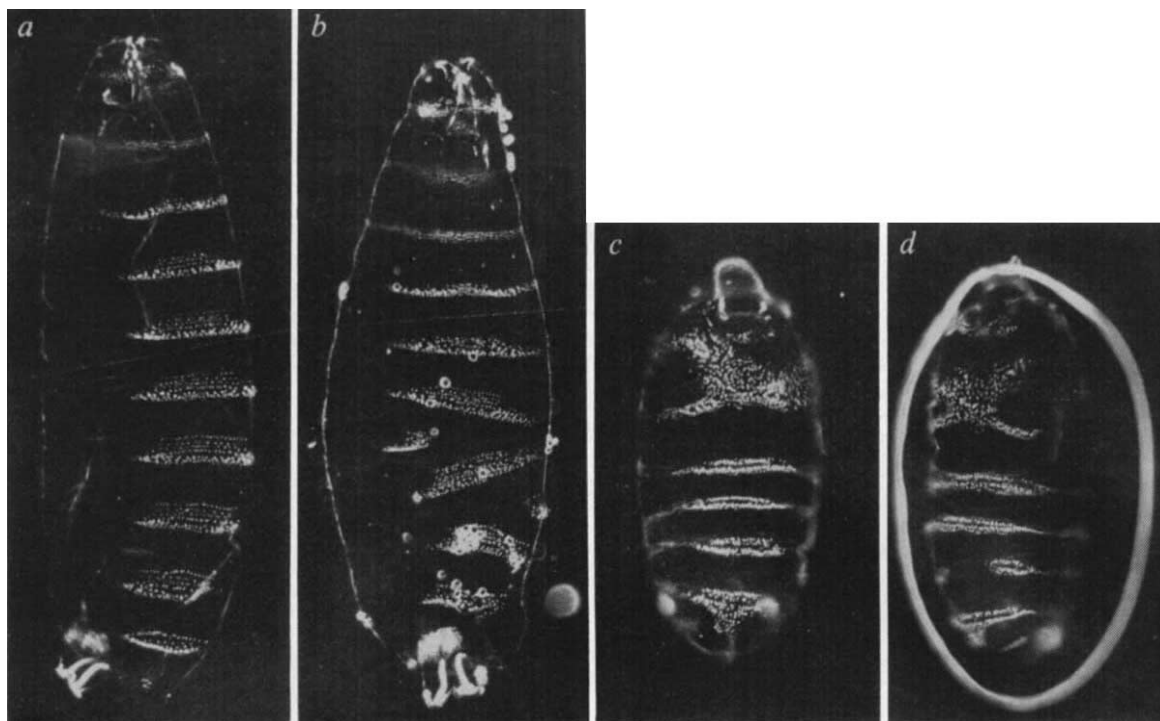


FIG. 3 Cuticular phenotypes of embryos from females with a homozygous double mutant *hb nos* germline. *a*, Zygotically rescued embryo, showing essentially the wild-type pattern, apart from the lack of the second thoracic segment, which is a frequent effect for the *hb*<sup>90</sup> allele under these conditions and thus proves the genetic identity of the embryo (compare legend to Table 1). *b*, Zygotically rescued embryo, showing defects in the fourth and sixth abdominal segment, which are the most sensitive segments for partial lack

of function of the posterior gene system<sup>9,11</sup>. *c*, Embryo with the combined maternal and zygotic *hb* phenotype. Abdominal segments four to six appear normal, implying that the expected *nos* phenotype is completely rescued. *d*, Embryo as in *c*, but showing a disturbance of the sixth abdominal segment. The embryos of the type shown in *b* and *d* occurred with a frequency of about 1 per cent.

mechanistically very different from the spatial organization of the anterior region by the morphogenetic *bcd* protein gradient<sup>2-4</sup>.

The normal function of the maternal *hb* activity may be to suppress an abdominal segmentation pathway in the anterior region at an early stage of embryogenesis. This inference is supported by the fact that denticle fields of abdominal quality are formed in embryos which lack both the maternal and the zygotic expression of *hb*, and also in embryos carrying certain abnormal gain of function alleles of *hb* (ref. 14). These denticle fields are, however, not comparable with normally organized segments, which is probably due to an interference from the influence of the anterior pattern organizer, *bicoid*, in this region. The presence of the maternal *hb* protein in the anterior region and its absence in the posterior region leads formally to an early subdivision of the embryo into two fields. The anterior field will give rise to head and thorax, whereas the posterior field allows abdomen formation. The borderline between the two fields corresponds functionally to the borderline between the domains

of expression of the two homoeotic gene complexes—the *Antennapedia* (*Antp*) complex, which specifies the anterior segments and the *bithorax* complex, which specifies the posterior ones (reviewed in ref. 24). Various observations suggest that the *hb* protein may indeed be a direct repressor of *Ultrabithorax*<sup>25-27</sup>, a gene of the *bithorax* complex; less is known about the regulatory interactions between *Antp* and *hb*, although *hb* may have an activating effect on *Antp*<sup>28</sup>. The borderline between head and thorax on the one hand and the abdomen on the other, is a functionally significant borderline in insects. In short germ-band embryos, head and thorax are organized together at an early stage, whereas the abdominal segments develop sequentially by budding (reviewed in ref. 29). This later spatial organization is possibly not directly dependent on maternal factors, but may be entirely under the control of zygotic interactions. The data presented here suggest that the development of a long germ-band embryo, like the *Drosophila* embryo, may be governed by a similar mechanism. □

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# Chemical model for Viking biology experiments: implications for the composition of the martian regolith

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THE 1976 Mars Viking biology experiments were designed to detect life by observing the products of biochemical reactions. In the labelled-release (LR) experiments<sup>1-4</sup>, about 25 nmol of <sup>14</sup>C-labelled gases evolved when regolith samples were moistened with nutrient solution. About 22% of the products reabsorbed upon second injection. As a biological test the LR results were positive, although the reabsorption was not readily explained. In the gas-exchange (GEX) experiments, up to 800 nmol of O<sub>2</sub> gas was evolved when samples were humidified<sup>5,6</sup>, suggesting that the martian regolith might contain a strong chemical oxidant which caused the LR results. Several chemical models have been proposed<sup>7,8</sup> but no self-consistent explanation of all of the observations has been achieved. Here we propose a chemical model for these biology experiments in which the reactants are an inorganic nitrate salt, which has been partly photolysed by ultraviolet light, and a sparingly soluble metal carbonate such as calcite. The model reproduces the main effects seen, indicating that nitrates are present in the martian regolith as well as calcite (or some other carbonate with similar solubility).

A satisfactory chemical model should, with a single assembly of reagents, provide the correct amounts of oxidant and oxygen gas, have the same thermal stabilities as those observed on Mars, produce the correct chemical reaction kinetics and reproduce the LR reabsorption effect. The model should also incorporate the possibility of independently varying the relative magnitudes of the oxidation and oxygen-release reactions, as these were observed to differ at different sample acquisition sites. The conditions required to form the chemical reactant(s) must be consistent with other information about Mars and the reactant(s) must be stable in the ambient physical and chemical environment of Mars and must remain so during the course of the Viking experiments.

Two components of our model have their origins in other studies of the Viking results. Levine and Straat<sup>9</sup> demonstrated with soils analogous to those of Mars that the second injection reabsorption occurs because of a shift in chemical equilibria involving CO<sub>2</sub>(g), water and soil components. Equilibrium calculations (R.C.P., unpublished results) show that CaCO<sub>3</sub>(s) is necessary to produce the observed reabsorption and that the cell contents must be mildly basic. The analogue soil samples used by Levine and Straat contained 6–7 wt% calcite and had a pH of 7.2. We have chosen a reactant that is effective under those conditions. Other simulations<sup>5,6,10-15</sup> have not allowed for this chemical constraint. Nussinov *et al.*<sup>16</sup> postulated that photolysis has generated CO<sub>2</sub>(g) and O<sub>2</sub>(g) in micropores in the martian regolith and that opening of these pores by water vapour caused the GEX responses. This concept is incorporated in our model.

Ultraviolet photons and X-rays decompose inorganic nitrates to nitrites and oxygen<sup>17-21</sup>. The oxygen gas forms micropores in which it is trapped. It has been shown<sup>22</sup> that peroxonitrite ion is also generated from KNO<sub>3</sub>. Peroxonitrite ion is stable at room temperatures but is destroyed by thermal treatment. Other

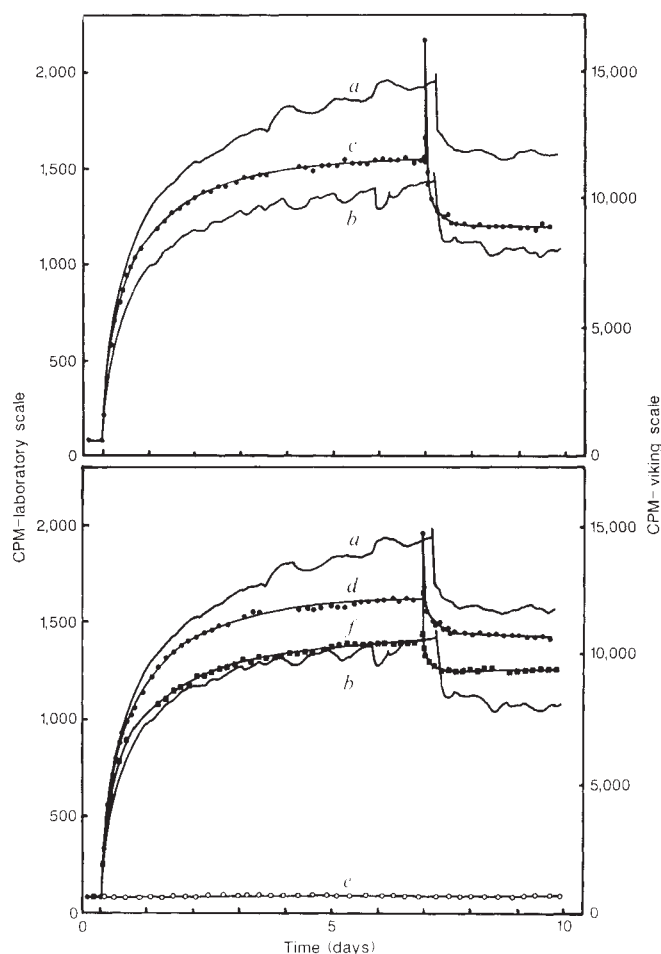


FIG. 1 Viking Lander labelled-release (LR) responses and laboratory simulations. *a*, VL2-cycle 1; *b*, VL1-cycle 1; *c*, 1.5 mmol KNO<sub>3</sub> irradiated for 225 min, 20 mg calcite and 2 mbar CO<sub>2</sub>. *d*, Same as *c* but with 6 mbar CO<sub>2</sub>. *e*, Same as *d* but also heat-treated for 3 hours at 148 °C. *f*, 2.0 mmol KNO<sub>3</sub> irradiated 60 min, 20 mg calcite and 6 mbar CO<sub>2</sub>. Data are plotted as counts per min (CPM).

METHODS. Cells for simulations were similar in internal dimensions and materials to those used in the Viking experiments<sup>31</sup> except that a manually controlled pyrex nutrient injector was used, the detector was a CaF<sub>2</sub>(Eu) scintillator window and access for vacuum manipulations was provided by a vacuum stopcock. Rigorous cleaning and passivating procedures similar to those employed by TRW Inc. in fabricating the Viking tests cells (F. S. Brown, personal communication) were used to avoid effects of the stainless-steel surfaces upon the kinetics of the reactions. The head end of the cell was maintained at 10 °C and the detector end at 22 °C, to reproduce the effects of the Viking LR test conditions. Heat treatments were performed by immersing the sample compartment in a molten wax bath. The nutrient was the same as that used in the Viking experiments except that the level of the labeling was 2 Ci mol<sup>-1</sup> of carbon rather than 8 Ci mol<sup>-1</sup>. Ethanol was added to bring the level of this impurity up to the 0.29 wt% level of the Viking nutrient (R.T., unpublished results). The response of the simulation cells was 2,000 CPM for 29 moles of labelled CO<sub>2</sub>(g) as opposed to 15,000 CPM for the Viking cells<sup>4</sup>, so that two ordinate scales have been used on graphs of the results, adjusted to make the results quantitatively comparable. The Viking timescale of sols has been converted to terrestrial days on the abscissae.

inorganic nitrates are expected to form peroxonitrite by photolysis. Peroxonitrite is a strong oxidizing agent in basic solutions but decomposes rapidly in acidic media<sup>23</sup>.

The quantities of nitrates and carbonates in the martian regolith are not known. The Viking X-ray fluorescence studies gave no direct information on nitrogen or carbon content. Spectroscopic studies<sup>24</sup> of 1971–72 dust storms indicating nitrate and carbonate levels of less than 5% are still considered valid<sup>25</sup>.

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However, there is known to be a flux of nitrogen oxide species to the surface from the atmosphere<sup>26</sup>, as a result of which some nitrates must have accumulated over geological time periods, especially considering that the pressure of  $N_2$  in the atmosphere was as much as 200 times greater in the past<sup>27</sup>. The trapping of  $NO_x(g)$  by the surface material has not received much study. We have shown that one suspected regolith component, magnesium sulphate, traps  $NO_x(g)$  chemically under desiccated low-pressure conditions, forming previously unknown mixed salts<sup>28</sup>. To represent surface nitrates, we used  $KNO_3$  in simulations, because cations have only secondary effects on the photolysis reactions and because it is not known precisely which inorganic nitrates would be formed from the flux of  $NO_x$  to the surface. The solar spectral distribution at the surface of Mars and its seasonal and latitudinal variations are known<sup>29</sup>.  $CO_2$  in the martian atmosphere absorbs ultraviolet radiation below 200 nm. Nitrate absorption bands begin at 330 nm (ref. 19) and extend into the X-ray region. For this work we have approximated the ultraviolet spectrum with a 253.7-nm source. A 225-min exposure in the laboratory provided about the same number of photons as a sample on the surface of Mars at central latitudes receives over the 200–300-nm range in 5 years.

The kinetics of the LR reactions can be used to test the accuracy of the simulations. We have found that the following empirical expression, with two exponential time constants  $t_1$  and  $t_2$ , linear slope  $K$  and normalization constant  $C$ , fits the Viking data for labelled gas release very well:

$$CPM = C[2 - \exp(-0.693t/t_1) - \exp(-0.693t/t_2) + Kt] + \text{background}$$

where CPM denotes the response in counts per minute. The data sets were signal-averaged to smooth the noise and fit to

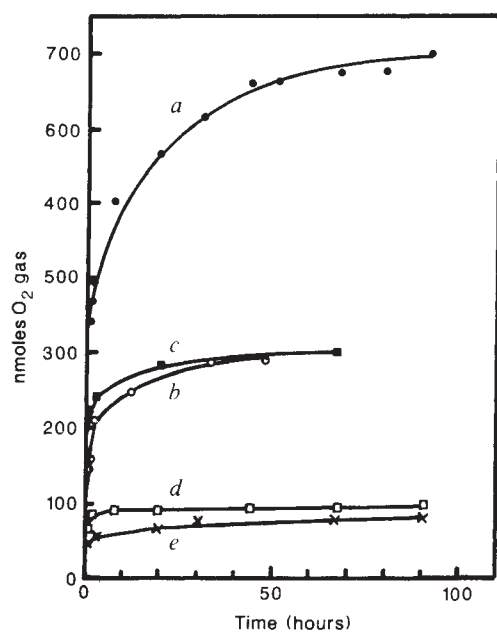


FIG. 2 Simulations of gas-exchange (GEX)  $O_2$  evolution effects. *a*, 3.0 mmol  $KNO_3$  irradiated for 225 min. *b*, Same as *a* but also heat-treated for 3 hours at 150 °C. *c*, 4.0 mmol  $KNO_3$  irradiated for 60 min. *d*, Same as *c* but also heat-treated for 3 hours at 150 °C. *e*, Blank, with no  $KNO_3$ . The Viking GEX samples were twice as large as the Viking LR samples; this ratio was reproduced in the simulations.

**METHODS.** The quantities of oxygen evolved in the GEX simulations were determined by pressure measurements in a pyrex vacuum system of calibrated volume, using a trap to remove water vapour. Degassed, distilled water (0.70 ml) was injected through a vacuum stopcock onto the sample, which was maintained at 10 °C. Heat treatments were performed by immersing the sample compartment in a molten wax bath.

the equation by least-squares statistical analysis<sup>30</sup>. The parameters derived from the first LR test at each of the landing sites are listed in Table 1. The two exponential components are clearly resolvable and they agree well between the two sites. The small linear terms are measurably different. The r.m.s. relative residuals,  $\sigma$ , are less than 1%. Differences in the magnitudes of the exponential terms could not be resolved; we have since found from simulations that the two components result from different oxidation rates of two nutrient species, which contribute approximately equally to the response (R.C.P., unpublished results).

Figures 1 and 2 show the results of simulations using our chemical model. LR responses of the same magnitude as the Viking results were obtained with 1.5 mmol of  $KNO_3$  irradiated for 225 min or 2.0 mmol irradiated for 60 min. The relative insensitivity to irradiation time results from the fact that the peroxonitrite is formed during early stages of irradiation, so that its concentration changes little during further irradiations. Thermal pretreatment causes the LR simulation response to disappear, as in the Viking experiments, because the peroxonitrite is decomposed by heating. The kinetics of the LR simulations agree well with the Viking results, showing both exponential components and similar time constants (Table 1).

TABLE 1 Kinetic parameters computed from Viking LR data and simulations

| Test                    | Kinetic parameters |              |                           |          |
|-------------------------|--------------------|--------------|---------------------------|----------|
|                         | $t_1$<br>(h)       | $t_2$<br>(h) | $K$<br>(h <sup>-1</sup> ) | $\sigma$ |
| Viking Lander 1—cycle 1 | 4.7                | 20           | 0.0018                    | 0.0079   |
| Viking Lander 2—cycle 1 | 4.4                | 20           | 0.0034                    | 0.0043   |
| Simulation <i>c</i>     | 3.8                | 21           | 0.0010                    | 0.0043   |
| Simulation <i>d</i>     | 4.0                | 24           | 0.00045                   | 0.0076   |
| Simulation <i>f</i>     | 3.1                | 23           | 0.0009                    | 0.0048   |

Simulations refer to equivalent designations in Fig. 1, and are as follows: *c*, 1.5 mmol  $KNO_3$  irradiated for 225 min, 20 mg calcite, 2 mbar  $CO_2$ ; *d*, 1.5 mmol  $KNO_3$  irradiated for 225 min, 20 mg calcite, 6 mbar  $CO_2$ ; *f*, 2.0 mmol  $KNO_3$  irradiated for 60 min, 20 mg calcite, 6 mbar  $CO_2$ .

If no calcite is used in the simulation, the kinetics change markedly,  $t_1$  and  $t_2$  falling to 0.5 hours and 16 hours. This is to be expected because such a change causes the medium to shift from being mildly basic to mildly acidic, under which conditions peroxonitrous acid decomposes at a significant rate.

The amount of  $O_2(g)$  released in GEX simulations varies over the same range as do the Viking results. The amount released is more dependent on irradiation time (Fig. 2*a* and *c*) than are the LR responses because the  $O_2(g)$  photolysis product increases in proportion to the radiation dose. The difference between the two photolysis processes suggests an explanation as to why the ratio of the LR to GEX responses varied for different sample acquisition sites. The GEX responses on Mars were reduced, but not eliminated, by heat treatment. The simulations in Fig. 2*b* and *d* show about the same degree of reduction. The rate at which water vapour is taken up by a powdered sample is sensitive to variables such as particle size and overall composition, which are not directly related to the reaction; therefore, the kinetics of the GEX reaction were not studied.

The second injection reabsorption of  $CO_2$  in the LR experiments was demonstrated in these studies. Under a partial pressure of 2 mbar  $CO_2$ , 24% of  $CO_2(g)$  was reabsorbed, whereas the reabsorption was only about 13% under 6 mbar  $CO_2$ . This pressure dependence of reabsorption conforms with expectations from chemical equilibrium calculations.

Both the peroxonitrite and the oxygen produced in inorganic nitrates by photolysis are stable with respect to long-term storage at the surface temperatures of Mars, so that they could have been accumulating in the martian regolith over geological time. A second sample for biological tests on Mars was taken from

beneath 'Notched Rock', that is, not from the exposed surface, in order to determine if the LR and/or GEX responses were the result of photochemical activity. The LR response was similar to that from a surface sample at the same site, whereas the GEX O<sub>2</sub> release was much lower. This could have been caused by a lower radiation exposure for the subsurface material, either by ultraviolet exposure at an earlier date when the material was on the surface or perhaps by exposure to other, more penetrating, types of radiation.

The fact that a large number of diverse features of the LR and GEX results can be reproduced with ultraviolet-irradiated potassium nitrate is strong evidence for the presence of nitrates in the martian regolith. The amounts of nitrate employed in these simulations correspond to 15–20 wt% of that in the regolith samples taken by Viking. However, until more is known about the formation of inorganic addition compounds under martian conditions and about the photochemistry of such compounds, conclusions cannot be drawn about the amounts or chemical forms involved. The simulation of the LR reabsorption effect, and the LR kinetics, require that calcite or another carbonate with similar solubility, such as argonite, be present. The pH of the martian regolith has been unknown previously; this model for the LR reaction shows that moistened regolith fines are mildly basic. □

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## Growth and anisotropic superconducting properties of Nd<sub>2-x</sub>Ce<sub>x</sub>CuO<sub>4-y</sub> single crystals

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**ELECTRON-DOPED copper oxide superconductors have recently been discovered in the system Ln<sub>2-x</sub>Ce<sub>x</sub>CuO<sub>4-y</sub>, where Ln is Pr, Nd or Sm<sup>1,2</sup>. Here we report the growth of single crystals of Nd<sub>2-x</sub>Ce<sub>x</sub>CuO<sub>4-y</sub>, and measurements of their anisotropic superconducting properties. A sharp superconducting transition is observed at 22.5 K in Nd<sub>1.84</sub>Ce<sub>0.16</sub>CuO<sub>4-y</sub>. The resistivity shows a**

**metallic temperature dependence both parallel and perpendicular to the basal plane. Applying a magnetic field perpendicular to the basal plane causes a parallel shift of the resistive transition curve to lower temperatures, as in conventional type II superconductors but unlike the hole-doped copper oxide superconductors. The Ginzburg–Landau coherence lengths estimated from the resistively defined upper critical magnetic field are 70.2 Å in the basal plane and 3.4 Å along the *c* axis, showing an anisotropy factor of 21. This value is much larger than that in La<sub>2-x</sub>Sr<sub>x</sub>CuO<sub>4-y</sub> despite their apparent structural similarities. Crystallographic differences from the hole-doped systems and the concomitant changes in electronic properties may offer clues to the role of the Cu–O planes in the microscopic pairing mechanism of high-temperature superconductors.**

Studies on copper oxide superconductors have shown that the parent compounds of (La, Sr)<sub>2</sub>CuO<sub>y</sub> (LSCO), YBa<sub>2</sub>Cu<sub>3</sub>O<sub>y</sub> (YBCO) and Bi–(Sr, Ca)–Cu–O (BSCCO) are antiferromagnetic. The doping of holes into the antiferromagnetic compounds results in a drop in the Neél (antiferromagnetic onset) temperatures (*T<sub>N</sub>*), and further doping leads to a metallic superconducting phase. The magnetic phase diagram of these oxides has been studied as a function of hole doping of the Cu–O layers, which have apical oxygen atoms. Muon spin resonance has revealed that the parent compound of the electron-doped system Nd<sub>2-x</sub>Ce<sub>x</sub>CuO<sub>4-y</sub> (NCCO) also shows an antiferromagnetic state at low temperatures<sup>3</sup>. It seems that magnetism is closely related to superconductivity in these superconductors also. A comparison of the physical properties of the p- and n-type copper oxide superconductors should give further insight into the high-*T<sub>c</sub>* mechanism.

We have grown single crystals from a CuO-rich non-stoichiometric solution of oxide starting materials. The flux method used here has been used previously to grow single crystals of other high-*T<sub>c</sub>* oxide compounds<sup>4</sup>. Nd<sub>2</sub>O<sub>3</sub>, CeO<sub>2</sub> and CuO powders with a purity of 99.99% were mixed in a concentration ratio of  $\frac{1}{2}$ Nd<sub>2</sub>O<sub>3</sub>:CeO<sub>2</sub>:CuO = 35:10:55 (atom %). This mixture was heated to 1,300 °C for 2 h, slowly cooled to 700 °C at a rate of 1.2 °C h<sup>-1</sup>, and then furnace-cooled to room temperature. As in the case of YBCO and BSCCO, crystals were found in cavities in the aggregate. They have a square plate-like shape with a well developed optically flat *c* plane. The average edge length of these crystals is several millimetres (the largest size is 10 × 10 × 0.3 mm), which is large enough to permit transport measurements. X-ray diffraction analysis showed that these crystals have a tetragonal T'-phase structure with lattice parameters *a* = 3.956 Å and *c* = 12.108 Å. The Ce content was determined by electron-probe microanalysis (EPMA), which showed that more than 12% (*x* = 0.24) Ce can be introduced into the system. Appropriate reducing conditions are necessary to obtain superconductivity in these samples. Bulk superconductivity was achieved by annealing at 950 °C for more than 8 h in an Ar atmosphere. Excessive reduction made the crystals transparent, with a blue tinge, and deficient in Cu. An excess of oxygen vacancies causes Cu evaporation from the Cu–O squares, probably as a result of the smaller coordination number of Cu ions relative to copper oxide superconductors that have apical oxygens.

The standard four-terminal electrical contacts were obtained by using silver wires of 50-μm diameter attached by a conducting silver paste to gold films evaporated on the *c* plane of the crystal. The dimensions of the sample were about 0.90 × 0.44 × 0.011 mm. The resistance was measured along the *a* axis in the basal plane and along the *c* axis. Temperatures were determined with a carbon-glass thermometer and corrected for magnetoresistance. A magnetic field of up to 12 tesla was applied with a superconducting magnet.

Figure 1 shows the temperature dependence of resistivity in the basal plane and along the *c* axis. In both directions, the temperature dependence exhibited typical metallic behaviour above *T<sub>c</sub>*. The resistance ratio *R*(300 K)/*R*(25 K) is 6.93 in the



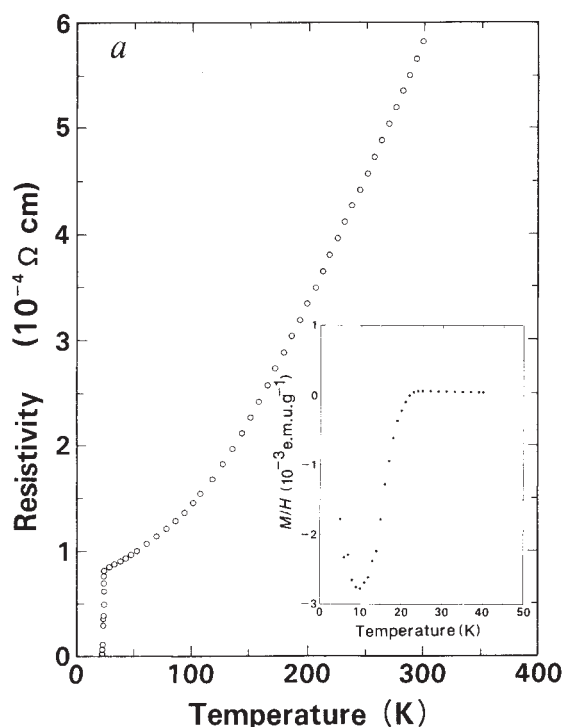
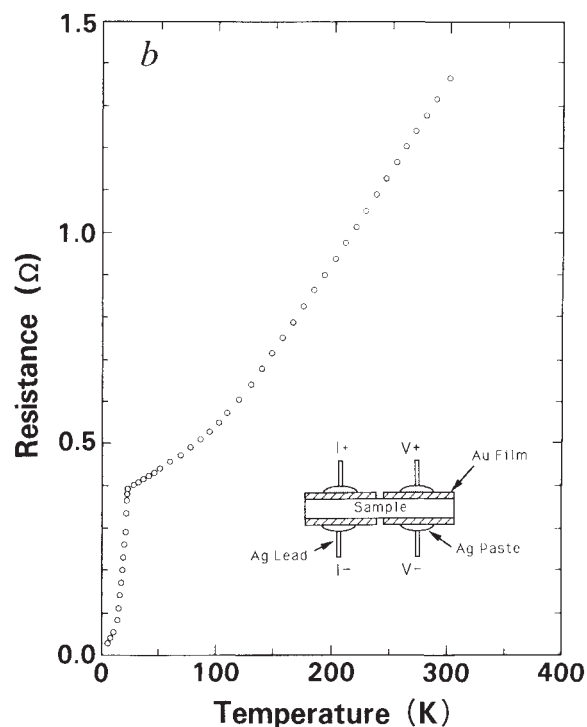


FIG. 1 Temperature dependence of resistivity for a  $\text{Nd}_{1.84}\text{Ce}_{0.16}\text{CuO}_{4-y}$  single crystal (a) in the basal plane and (b) along the  $c$  axis. Inset to a, Temperature dependence of the Meissner signal measured by cooling the



sample under a constant field of  $H = 50$  Oe. Inset to b, Electrode configuration for  $c$ -axis resistivity measurement.

basal plane and 5.17 along the  $c$  axis. The temperature coefficient of resistivity,  $d\rho/dT$ , which is defined for the linear portions of Fig. 1a and b, is  $2.49 \mu\Omega \text{ cm K}^{-1}$  along the basal plane and  $1.73 \text{ m}\Omega \text{ cm K}^{-1}$  along the  $c$  axis. A large Meissner signal of more than 25% of the ideal value is observed (inset to Fig. 1a), which indicates bulk superconductivity.

To measure the resistivity along the  $c$  axis, electrodes were attached as shown in the inset of Fig. 1b. This method involves some ambiguity because of the indefinite sample cross-section; resistivity was estimated by regarding the area of the gold film as the sample cross-section. The superconducting transition begins at 22.5 K and the zero-resistance state persists up to 21 K. For measurements along the  $c$  axis, zero resistance was not observed even at 4.2 K. The broad and incomplete transition may be ascribed to the inhomogeneous oxygen distribution along the  $c$  axis. In general a crystal surface is reduced to a greater extent than the interior. Even in a sample with a smoothly varying oxygen distribution and a broad resistive transition along the  $c$  axis, a sharp superconducting transition is observed in the resistivity along the basal plane, implying that current flows in highly reduced superconducting regions. For this reason great care must be taken in measuring the resistivity along the  $c$  axis; anisotropy of the resistivity is very sensitive to inhomogeneities in the oxygen distribution.

Figure 2 shows the temperature dependence of the Hall coefficient  $R_H$  in a sample with Ce concentration of  $\sim 1.26 \times 10^{21} \text{ cm}^{-3}$ . The carrier density for this composition, derived from  $R_H$ , is  $1.18 \times 10^{21} \text{ cm}^{-3}$ , which is almost the same as the Ce concentration. The number of carriers seems therefore to be controlled only by Ce-doping without reduction, although the effects of reduction in this system are not yet known. As temperature decreases,  $R_H$  increases to a maximum at 100 K. This is similar to the behaviour observed in superconducting samples of the YBCO and LSCO systems<sup>5</sup>. The electron character of the carriers is also indicated by measurements of the Seebeck coefficient, which has an average negative value of about  $-1 \mu\text{V K}^{-1}$  and shows typical metallic temperature dependence.

Figure 3 shows the temperature dependence of resistivity as a function of an applied field  $H$  parallel and perpendicular to the Cu-O plane. Parallel to the basal plane, the transition broadens with increasing field, as is frequently observed in single crystals of YBCO and BSCCO. This observation has been explained in terms of flux creep with a short coherence length  $\xi$  in the two-dimensional Cu-O planes. For  $H$  parallel to the  $c$  axis, however, this broadening of the transition tail becomes less pronounced, and instead one observes parallel shifts which

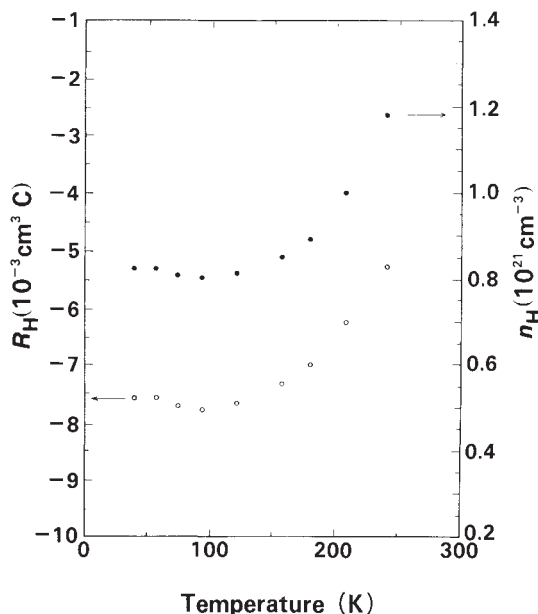


FIG. 2 Temperature dependence of Hall coefficient and carrier density for a  $\text{Nd}_{1.88}\text{Ce}_{0.12}\text{CuO}_{4-y}$  single crystal.

are typical of conventional isotropic type II superconductors. In a field of more than 6.5 T, the resistivity increases with decreasing  $T$  below 10 K. In addition, significant magnetoresistance is observed for temperatures of up to at least 60 K. When the field is applied along the basal plane, however, no such magnetoresistance is observed. Significant magnetoresistance over such a wide temperature range has not been observed for other copper oxide superconductors.

As Malozemoff *et al.*<sup>6</sup> have pointed out, when shifts of the transition tail occur for the field-induced resistive transition, determining  $H_{c2}$  from this transition may lead to a serious underestimation as a result of extensive flux creep, particularly if  $T_0(H)$  is defined at  $\rho = 0$ . One way to avoid this difficulty is to define  $T_c$  at the midpoint of the transition or at some large fraction of normal resistivity  $\rho_N$ . Figure 4 shows  $H_{c2}(T)$  when defined as the field at which resistivity falls to half the normal value (defined by extrapolation from temperatures above  $T_0$ ). The  $H_{c2}(T)$  curves for  $H$  perpendicular to the  $c$  axis seem to be diverging, which may be ascribed to flux creep. For  $H$ , parallel to the  $c$  axis, the  $H_{c2}(T)$  curve retains a positive curvature near  $T_c$  and saturates to a value of 6.7 T as  $T$  approaches zero. This temperature dependence resembles the behaviour observed in two-dimensional superconductors such as TaS<sub>2</sub> (ref. 7). From tangents to the curves at  $T = 20.5$  K for a parallel field and  $T = 10$  K for a perpendicular field, we obtain  $-dH_{c2}/dT$  values of 0.43 T K<sup>-1</sup> along the  $c$  axis and 8.85 T K<sup>-1</sup> along the basal

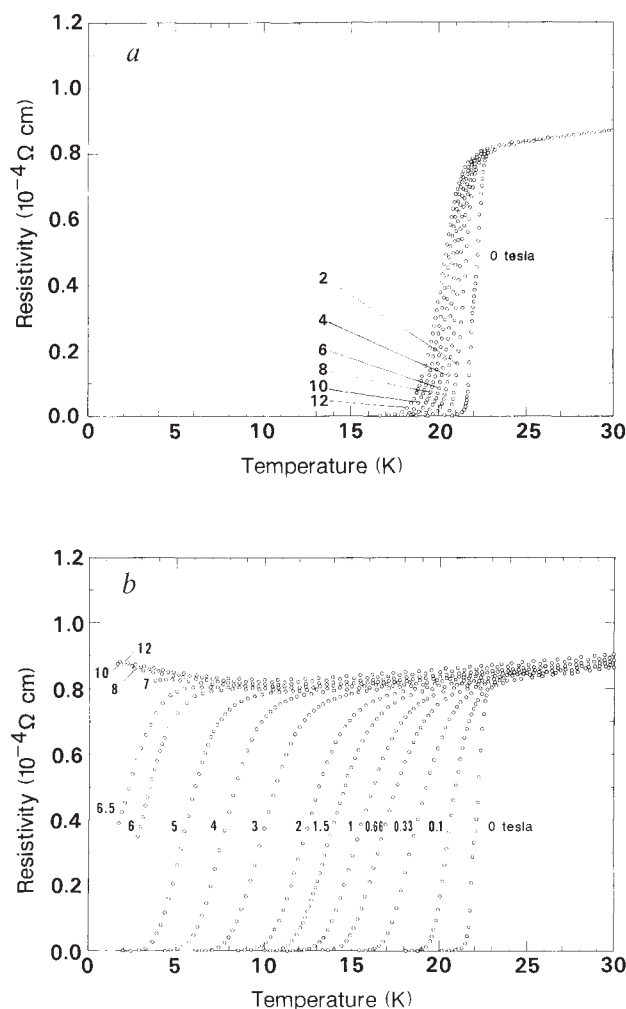


FIG. 3 Temperature dependence of resistivity for a Nd<sub>1.84</sub>Ce<sub>0.16</sub>CuO<sub>4-y</sub> single crystal under a magnetic field of up to 12 T (a) parallel to and (b) perpendicular to the Cu-O plane.

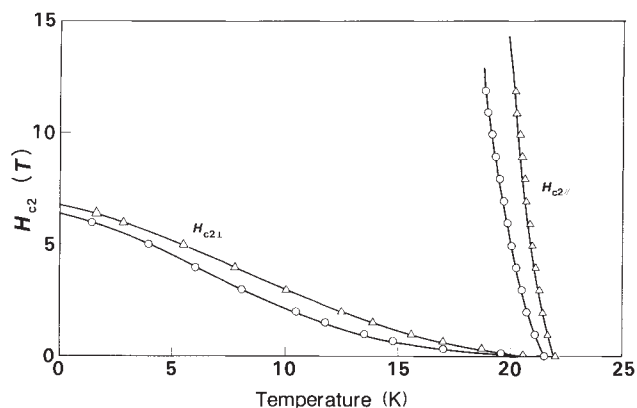


FIG. 4 Temperature dependence of resistively defined  $H_{c2}$  (parallel and perpendicular to the axis) for a Nd<sub>1.84</sub>Ce<sub>0.16</sub>CuO<sub>4-y</sub> single crystal.  $H_{c2}$  is defined as the field for which resistivity becomes zero (circles) or half (triangles) the normal resistivity.

plane. According to the Werthamer-Helfand-Hohenberg (WHH) theory for type II superconductors,  $H_{c2}(0)$  may be estimated from  $H_{c2}(0) = -0.69 T_c (dH_{c2}/dT)_{T=T_c}$  which in this case gives  $H_{c2}$  values of 6.69 T and 137 T for the  $c$  axis and basal plane respectively. The former value is in quite good agreement with the experimental (saturated) value. Using these values, the Ginzburg-Landau coherence length along the  $c$  axis at 0 K is estimated to be 3.4 Å and that in the basal plane is 70.2 Å. This latter value is fairly large compared with other copper oxide superconductors. We stress, however, that these estimates of  $\xi$  are lower limits only.

These results suggest that NCCO has pronounced anisotropy in its electronic structure. The anisotropy factor for  $H_{c2}$  with respect to the  $c$  axis is estimated to be 21, which value is considerably larger than those of LSCO (a factor of 13 (ref. 8) and YBCO (a factor of 6 (ref. 9)). The larger anisotropy of NCCO may be due to the lack of apical oxygen atoms in the Cu-O planes.

Thus the anisotropic properties of NCCO single crystals have been studied. The temperature dependence of resistivity shows typical metallic behaviour both along and perpendicular to the basal plane. This system exhibits significant anisotropy of resistivity and critical field  $H_{c2}$  with respect to the  $c$  axis. The 'parallel shift' behaviour of the tail of the field-induced resistive transition provides a good basis for defining  $H_{c2}$ . We find that the coherence length along the basal plane is 70 Å, a value that is quite large compared with that in LSCO, a hole-doped system. The parallel shift in the transition tail may be related to the large coherence length. The lack of apical oxygen may limit electronic conduction along the  $c$  axis, if this conduction occurs via Cu-O hybridized orbitals. A homogeneous oxygen distribution should be realized for further studies of anisotropy. Whether or not a large-scale magnetic fluctuation also exists in this electron-doped system remains an important question, to resolve which we are now growing larger single crystals suitable for neutron-scattering experiments. □

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# New transformations between crystalline and amorphous ice

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AMONG the unusual physical properties of  $\text{H}_2\text{O}$ , its behaviour at low temperatures and high pressures is particularly anomalous and incompletely understood<sup>1-3</sup>. Mishima *et al.*<sup>1,2</sup> have shown that crystalline ice- $\text{I}_h$  transforms at 1 GPa and 77 K to a high-density amorphous (hda) form, which exhibits unusual thermal properties. With the exception of volume displacement measurements, however, no *in situ* studies of this high-pressure polymorphism have yet been reported<sup>3</sup>. Using high-pressure optical and spectroscopic techniques, we have observed directly the ice- $\text{I}_h \rightarrow$  hda-ice transformation in a diamond-anvil cell, and have examined the stability of the amorphous form as functions of pressure and temperature. We demonstrate that hda-ice transforms abruptly at 4 GPa and 77 K to a crystalline phase close in structure to orientationally disordered ice-VII and to a more highly ordered (ice-VIII-like) structure at higher temperatures. This is the first time that an amorphous solid is observed to convert to a crystalline solid at low temperatures by compression alone. The results are in excellent agreement with recent theoretical predictions<sup>4</sup> for  $\text{H}_2\text{O}$  and support the accuracy of recently developed interatomic potentials for its ordered and disordered hydrogen-bonded phases. Phase transitions of this type may be relevant on icy planetary satellites, and there may also be implications for the high-pressure behaviour of silica.

Vibrational spectroscopy has long been used as a structural probe of  $\text{H}_2\text{O}$  phases because of the information it provides on the state of intramolecular and intermolecular interactions in the material<sup>5</sup>. Recent studies have shown that high-pressure spectroscopic techniques can be used to reveal theoretically predicted, but non-quenchable, pressure-induced structural transformations in glassy materials such as amorphous  $\text{SiO}_2$  (refs 6, 7). Here we used vibrational Raman scattering to probe structures and identify phase transformations *in situ* using diamond-anvil-cell techniques<sup>8</sup>. Cylindrical samples (250  $\mu\text{m}$  diameter  $\times$  250  $\mu\text{m}$  thickness), consisting of doubly distilled, deionized water confined by the stainless-steel gaskets of the cell, were used. The pressures were determined using the ruby fluorescence scale<sup>9</sup> corrected for temperature dependence of the zero-pressure wavelength of the  $R_1$  fluorescence band<sup>10,11</sup>. Raman spectra of the crystalline ices  $\text{I}_h$ ,  $\text{I}_c$ , II, III, VI, VII and VIII and the low- and high-density amorphous forms were obtained under variable pressure and temperature conditions (R.J.H., L.C.C. and H.K.M., manuscript in preparation) and compared with available X-ray diffraction data<sup>12,13</sup>. Transformations were readily identified *in situ* by the characteristic low-frequency lattice mode and high-frequency O-H stretching Raman bands for each phase. Three series of experiments were performed as functions of pressure between 0.1 MPa (1 bar) and 30 GPa and temperature between 77 K and 300 K.

In the first set of experiments, we studied ice under isothermal compression and decompression at 77 K to maximum pressures of up to 30 GPa. Representative *in situ* Raman spectra of the high-frequency O-H stretching region are shown as a function of pressure in Fig. 1. The spectrum measured at 0.3 GPa is readily identified as that of ice- $\text{I}_h$ . At 0.8 GPa, however, a broadened band at  $\sim 3,100\text{ cm}^{-1}$  begins to grow at the expense of the sharp ice- $\text{I}_h$  peaks. These changes in the Raman spectrum are accompanied by marked textural changes in the sample with increasing pressure. At  $\sim 0.5$  GPa, extensive fracturing of the sample occurs, and the sample becomes turbid by a pressure of 1.0 GPa, with the new phase appearing along fracture faults. The mixed phase causes strong scattering of visible light up to  $\sim 1.5$  GPa, whereupon the sample becomes homogeneous again.

Previous piston-cylinder displacement experiments indicate that ice-I collapses to form an amorphous form in the pressure interval 1.0–1.5 GPa (ref. 1). The high degree of light scattering arises from the large  $\Delta V$  of the  $\text{I}_h \rightarrow$  hda transformation<sup>1</sup>. The high-frequency Raman spectrum of the homogeneous sample above 1.5 GPa contains a single asymmetrically broadened band which decreases in frequency with increasing pressure (for example, it occurs at  $3,065\text{ cm}^{-1}$  at 3.5 GPa). The spectrum is similar to that measured for the material quenched from 2–4 GPa to 0.1 MPa in our experiments and to that obtained by Klug *et al.*<sup>14</sup>, who identified it as hda-ice. Our *in situ* measurements thus demonstrate that an amorphous form is produced directly from ice- $\text{I}_h$ . These measurements provide the first evidence that the high-density amorphous form previously studied in quench experiments does not result from retrograde vitrification of a non-quenchable crystalline high-pressure phase.

With further increase in pressure to  $\sim 4$  GPa, a transformation boundary appeared at the sample-gasket interface and propagated toward the centre of the sample with increasing pressure. The sample transformed completely when the minimum pressure reached 4.6 GPa. The spectrum measured at this pressure consists of an intense, well resolved peak at  $3,179\text{ cm}^{-1}$ , with weaker features at approximately  $3,300$  and  $3,380\text{ cm}^{-1}$  (Fig. 1). With the exception of shifts of the peaks to lower frequency with increasing pressure, the spectrum remains largely unchanged to pressures above 30 GPa. The well resolved peaks in the spectrum in both the low-frequency lattice-mode and high-frequency O-H stretching regions suggest increased ordering in the structure. In fact, the spectrum of the new phase measured above 4 GPa is indistinguishable in both regions from that of a crystalline

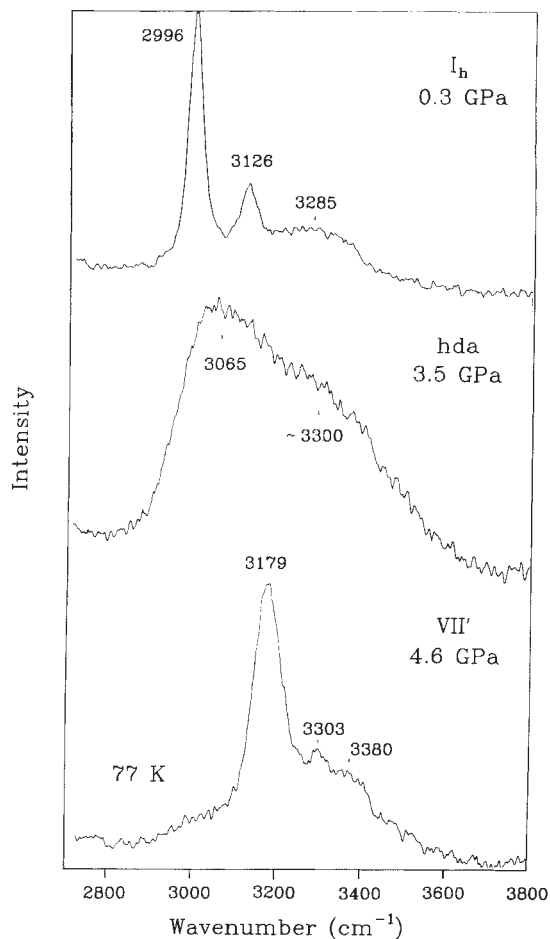


FIG. 1 *In situ* Raman spectra of the high-frequency O-H stretching region of ice as a function of pressure at 77 K.

ice-VII-like phase produced from compression of ice-VI at 77 K (R.J.H., L.C.C. and H.K.M., manuscript in preparation). The equilibrium phase under these conditions is, however, ice-VIII, with ice-VII the stable phase at higher temperatures. The relative intensities of the O-H stretching bands and splittings of the low-frequency lattice modes measured for ice-VIII<sup>15,16</sup> are not observed for the new phase. The crystal structures of ice-VII and VIII are closely related: the former has a cubic structure (space group  $Pn3m$ ) and proton disorder whereas the latter is proton-ordered with a tetragonal structure ( $Pa2_1/nmc$ )<sup>13</sup>. If the crystalline phase produced from hda at 77 K is a disordered (or partially disordered) form of ice-VIII, it is possible that hda-ice will transform to a more ordered (ice-VIII-like) structure at higher temperatures.

In a second series of experiments, we isothermally compressed ice- $I_h$  at temperatures above 77 K to examine this temperature dependence. In these measurements we also studied the maximum temperature at which pressure-induced amorphization of ice- $I_h$  occurs in the diamond-anvil cell. We were able to observe the crystalline-amorphous-crystalline transformation sequence at temperatures as high as  $\sim 150$  K (on timescales of several minutes). At higher temperatures, and over longer time-scales, the  $I_h \rightarrow$  hda transformation appeared to be occluded by the equilibrium  $I_h \rightarrow$  II transition. Increasing temperature tends to lower the hda-ice crystallization pressure; at 150 K, for example, the transformation is complete by 2.4 GPa. With increasing temperature between 77 and  $\sim 150$  K, the hda-ice formed from ice- $I_h$  crystallizes in a phase that has a spectrum identical to that of ice-VIII under the same conditions of pressure and temperature. Moreover, the crystalline phase produced from hda-ice at 77 K also transforms to ice-VIII with increasing temperature at high pressure. We therefore conclude that the phase produced at the lower temperatures of  $\sim 77$  K has a structure similar to the frozen-in disorder of ice-VII and that crystallization of hda-ice into a fully ordered solid is kinetically hindered in this temperature range. This low-temperature form is tentatively identified as ice-VII'.

In a third set of experiments, we studied the thermal stability

of hda-ice at low and high pressure. In particular, we were interested in the possibility of preserving hda-ice under pressure at room temperature. The amorphous phase was formed by compression of ice- $I_h$  at 77 K to a pressure of 3 GPa and was warmed at  $\sim 1$  K  $\text{min}^{-1}$ . When the temperature reached 160 K, a transformation boundary was observed to move from the edge of the sample toward the centre. The spectrum of the material at  $\sim 160$  K and 2.6 GPa is shown in Fig. 2. The spectrum of the higher-temperature phase is identical to that of ice-VIII, the stable equilibrium phase under these conditions. The ice-VIII was stable upon further heating to near 280 K, at which point it transformed to ice-VII (indicating some metastability of VIII in the stability field of VII). This experiment was then repeated at a series of pressures. We were unable to recover the hda form at temperatures above  $\sim 160$  K. This upper temperature bound seems to be pressure-insensitive: above 150–160 K, amorphous  $\text{H}_2\text{O}$  crystallizes as ice-VIII, VI or I (ref. 1), depending on the pressure.

The pressure- and temperature-induced transformations in ice observed in this study are summarized schematically on the phase diagram of  $\text{H}_2\text{O}$  in Fig. 3. Recent studies have shown that pressure-induced amorphization of low-pressure phases (and of high-pressure phases on decompression) can be understood in terms of thermodynamic and elastic properties of the metastable phases<sup>17–20</sup>. Considering first the  $I_h \rightarrow$  hda transformation, we note that the shear strength of ice- $I_h$  at 77 K decreases with increasing pressure above 0.25 GPa (ref. 21). Extrapolation of these data indicates that the shear strength of ice- $I_h$  may vanish below 1 GPa (77 K), that is, near the onset of amorphization (and not the  $I \rightarrow$  II transformation, as originally interpreted<sup>21</sup>). This behaviour suggests that the amorphization arises from a shear-mode softening, as proposed previously<sup>19</sup> for crystalline  $\text{SiO}_2$ . The driving force for the hda  $\rightarrow$  VIII(VII') transformation can be understood from an examination of the relative densities of the crystalline and amorphous forms measured at 0.1 MPa (refs 1, 2). The density of ice-VIII exceeds that of hda at 0.1 MPa by 27% (density  $\rho_0 = 1.49$  and  $1.17$   $\text{g cm}^{-3}$ , respectively<sup>1,13</sup>). The density of ice-VII, which is non-quenchable, is close to that of

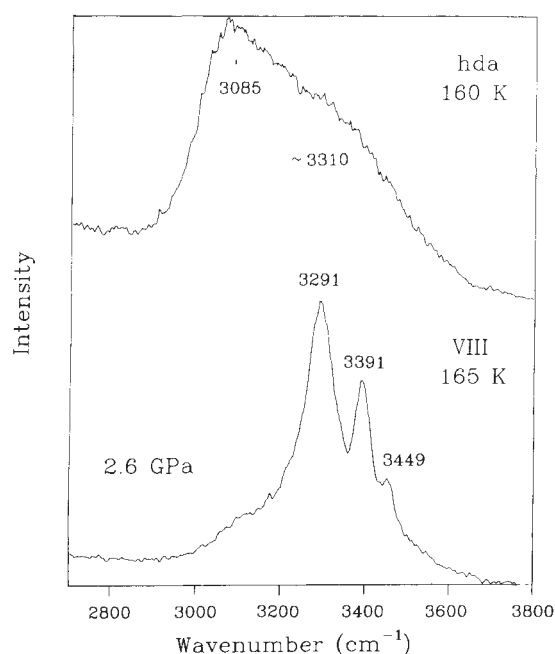


FIG. 2 *In situ* high-pressure Raman spectra showing the crystallization of ice-VIII from hda-ice at  $\sim 160$  and 2.6 GPa. The 3,291-, 3,391- and 3,449- $\text{cm}^{-1}$  peaks of ice-VIII are assigned to  $A_{1g}$ ,  $E_g$ , and  $B_{1g}$  symmetries, respectively<sup>12</sup>. The weak shoulder at  $3,100$   $\text{cm}^{-1}$  in the lower spectrum is due to residual untransformed hda-ice, which disappeared at higher temperatures.

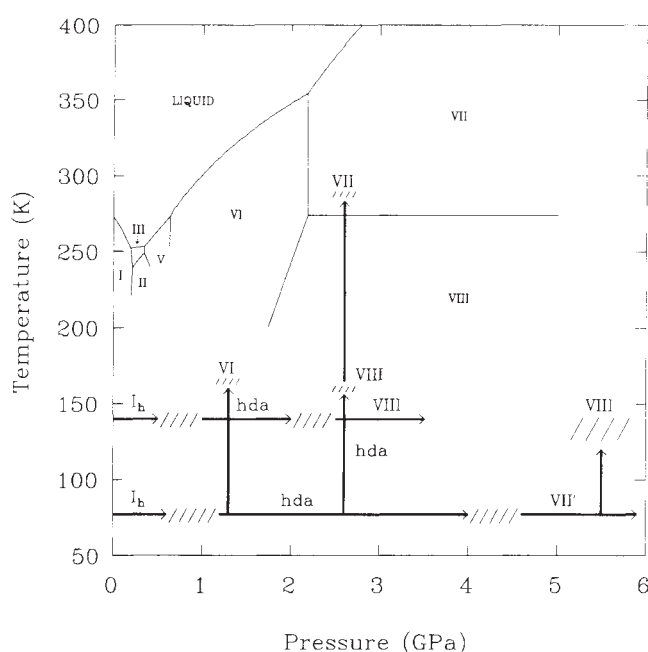


FIG. 3 Transformations observed in the present experiments superimposed on the equilibrium phase diagram of  $\text{H}_2\text{O}$ . The pressure-temperature ( $P$ - $T$ ) trajectories are indicated schematically by the horizontal and vertical arrows. The hatched areas indicate mixed-phase regions found to be stable over the timescale of the experiments (typically 3–5 h) for a given  $P$ - $T$  path.

VIII<sup>12,13</sup>; recent high-pressure X-ray diffraction measurements indicate that the basic oxygen substructure of this phase is stable to ultrahigh pressures (to at least 128 GPa) at 300 K. As metastable transformations are partially controlled by the kinetic factors, it is anticipated that cooling below 77 K may significantly increase the pressure of the hda  $\rightarrow$  VIII transformation. Lower-temperature studies could also be used to reveal the extent to which the I<sub>h</sub>  $\rightarrow$  hda transformation is controlled by thermally activated versus diffusionless (for example, elastic-instability) mechanisms. The transformation pressures and temperatures are likely to be affected to some degree by the state of stress in the sample and by the nucleation sites. In each experiment, for example, crystallization was observed to begin at the sample-gasket interface, which is also where the sample pressure is highest for a given load. In addition, we were able to lower the pressure of the hda  $\rightarrow$  VIII transformation at 77 K to 2.4 GPa under conditions in which small amounts of ice-II were formed in the ice-I<sub>h</sub> sample while cooling under pressure.

The observation of pressure-induced crystallization of hda-ice is in excellent agreement with the theoretical predictions of Tse and Klein<sup>4</sup> using constant-pressure molecular dynamics with the TIP4P H<sub>2</sub>O potential<sup>22</sup>. They predicted that hda-ice will transform under pressure to ice-VIII and further proposed that the high-pressure phase is partially proton-disordered at lower temperatures (80 K in their simulations). The predicted crystallization pressure of  $\sim 5$  GPa is in quantitative agreement with experiment; in addition they closely reproduced the pressures and temperatures of the I<sub>h</sub>  $\rightarrow$  hda and hda  $\rightarrow$  lda transitions, respectively. This agreement between theory and experiment indicates that the form of the interatomic potential for both ordered and disordered phases is accurately represented by the TIP4P model, a remarkable result in view of the historical difficulties associated with the refinement of structure-independent interatomic potentials for H<sub>2</sub>O. The recent high-pressure diffraction and spectroscopic measurements on ice indicate dramatic changes in molecular bonding at higher pressures (that is, above 30–40 GPa) (refs 12, 16 and R.J.H., L.C.C. and H.K.M., manuscript in preparation). At these pressures further refinements in the potentials are required to represent accurately the physical properties of H<sub>2</sub>O (refs. 12, 23).

Finally, there are several implications of the present work for planetary geology. Gaffney and Matson<sup>24</sup> have proposed that high-pressure crystalline phases can be produced by meteorite impact on the surfaces of icy satellites and that these phases may remain in planetary regoliths as a result of the estimated low average surface temperatures (<120 K). The low-temperature static-compression data indicate that hda-ice could also form by meteorite impact (as well as laboratory shock-wave studies of low-temperature ice<sup>25</sup>). If shock pressures exceed 4 GPa and/or post-shock temperatures exceed 150 K, however, the present data indicate that any hda-ice that is formed will quickly transform to the equilibrium crystalline phase. If temperatures remain below 150 K, on the other hand, any amorphous ice quenched in such processes may remain in the regolith for long periods of time, although transformations to lower-density amorphous forms may occur on release of pressure<sup>1</sup>. Our results for ice also suggest the need to examine possible pressure-induced crystalline-amorphous-crystalline transformations in silicate minerals that vitrify under pressure. For example, it is possible that stishovite can be formed at lower temperatures than has been previously thought (that is, at 300 K) by pressure-induced crystallization of densified amorphous SiO<sub>2</sub> produced from compression of either vitreous silica<sup>6</sup> or quartz<sup>19</sup>. □

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## Molecular layering in the spreading of wetting liquid drops

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THE details of the mechanisms involved in the spreading of liquids on solid substrates are still poorly understood, partly because dominant phenomena may occur in the range 0 to 100 Å from the substrate. Experimental investigation at the molecular level should provide information on the basic physical process involved. Here we present the results of studies on the time-dependent thickness profile of tiny drops spreading completely on a smooth surface (a silicon wafer). We use simple liquids which lack any orientational or structural order in the bulk. Using ellipsometry, an optical technique that provides measurements of thickness with sub-molecular resolution, we show that there is strong, dynamic structuring of the fluid near the solid surface, in that the spreading droplet advances as a series of distinct molecular layers. These observations may provide a clue to the nature of the molecular interactions or fluid flow phenomena involved in the spreading process.

There are two possible situations for a droplet resting on a solid surface. For partial wetting, there exists a macroscopically observable contact angle  $\theta$  between the liquid droplet and the surface. The contact angle is given by Young's equation:  $\cos \theta = (\gamma_{sv} - \gamma_{sl})/\gamma_{lv}$ , where  $\gamma_{ij}$  is the interfacial tension (free energy per unit area) of the (i, j) interface, with l, s and v denoting liquid, solid and vapour, respectively. This corresponds to an equilibrium situation, in that the droplet does not move with time. For complete wetting, the contact angle defined by Young's equation is zero, so that a liquid droplet will tend to spread when deposited on the surface, the initially non-zero contact angle tending towards its equilibrium value with time.

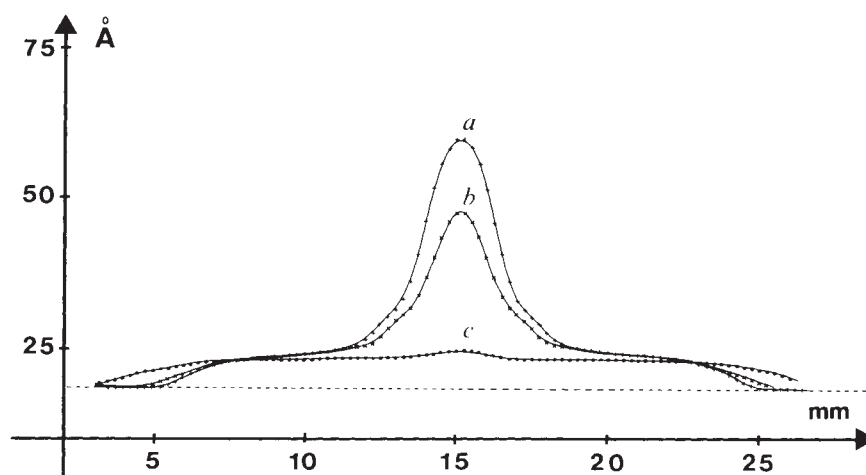
Despite the fact that practical applications of wetting are numerous (for example, lubrication, coatings, tertiary oil recovery and adhesion), some important aspects of the phenomenon are still incompletely understood; in particular, studies of the dynamics of the process have begun only recently. The physics of an advancing edge of a liquid drop on a substrate depends both on macroscopic scales ( $\geq 1 \mu\text{m}$ , for which the apparent contact angle  $\theta$  is the relevant quantity, and fluid mechanics plays a role) and on 'microscopic' scales (of the order

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FIG. 1 Ellipsometric thickness profile of a PDMS drop spreading on a silicon wafer. The volume of the drop is  $1.9 \times 10^{-4} \mu\text{l}$ . The thickness measured far from the drop is non-zero, because of a 17.2-Å-thick surface layer of silicon oxide. This baseline thickness (dashed line) corresponds to the bare solid on which the liquid spreads. The times  $\tau$  after deposition of droplet are *a*,  $\tau=47$  h, *b*,  $\tau=56$  h and *c*,  $\tau=96$  h.



of a few Å, where details of the liquid-substrate molecular interaction become important). The macroscopic process of spreading may be driven by interfacial phenomena at the molecular scale. For complete wetting, it has been known for some time that a 'precursor film' of liquid, of sub-micrometre thickness, spreads ahead of the macroscopically observable drop<sup>1</sup>. The understanding of the shape and dynamics of such films is at present an active field of research<sup>2-8</sup>. We have performed detailed measurements of drop profiles for two different liquids, and have found dynamic (that is, time-dependent) structuring of the precursor film at a molecular level.

In our experiment, the film thicknesses are measured by spatially and time-resolved ellipsometry<sup>9,10</sup>. The substrates are [111] silicon wafers designed for electronic components, which were cleaned by ultraviolet irradiation under ozone<sup>11</sup> and are known to have near molecular smoothness<sup>12</sup>. The liquids (refractive index  $n=1.4$ ) are deposited on the substrate ( $n=3.9-$

0.02i), and the thicknesses are determined from the differences between measurements without liquid and measurements after deposition. The spot of measurement is  $200 \mu\text{m}$  by  $1 \text{ mm}$ , and the stability of the thickness measurement is typically  $\pm 0.1 \text{ Å}$  per day (for a rate of one measurement per 10 ms).

Our experiment is not performed under ultrahigh vacuum, but rather at room temperature and in a room-pressure enclosure designed to reduce contamination (using a teflon sample holder, and a continuous flow of dry filtered nitrogen gas). Residual contamination (apparent as a continuous increase in thickness when no liquid is present) was found to contribute errors of the order of  $0.2 \text{ Å}$  per week.

The wetting fluids are PDMS (polydimethylsiloxane, a polymer of low molecular weight (with no specific polymeric effect),  $M_p=2,400$ , polydispersity index  $I_p=1.7$  and a viscosity  $\eta=2 \times 10^{-2} \text{ Pa s}$  (courtesy of Rhone Poulenc)) and tetrakis(2-ethylhexoxy)-silane (molecular weight  $M_w=545$ ,  $\eta=6.8 \times 10^{-3} \text{ Pa s}$ ).

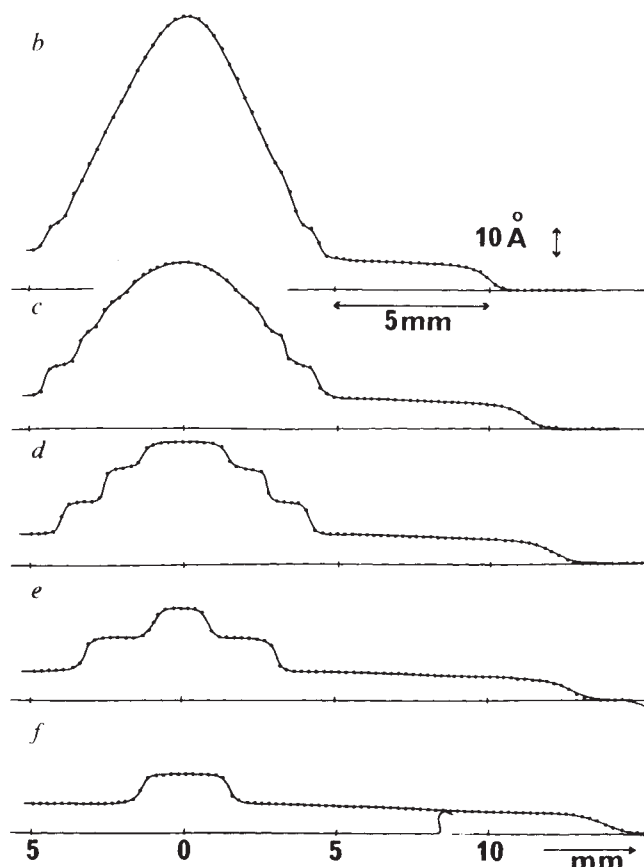
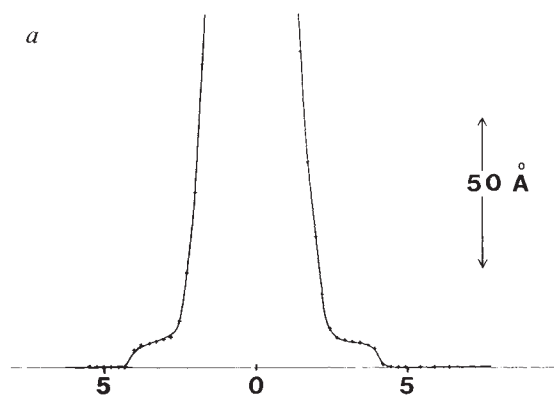


FIG. 2 Ellipsometric thickness profile of a tetrakis(2-ethylhexoxy)-silane drop spreading on a silicon wafer (volume of the droplet  $=4.7 \times 10^{-4} \mu\text{l}$ ). The base line (silicon oxide thickness of the substrate) has been subtracted: zero thickness corresponds to zero thickness of fluid. Time  $\tau$  after deposition of droplet: *a*,  $\tau=8$  h, *b*,  $\tau=71$  h, *c*,  $\tau=83$  h, *d*,  $\tau=118$  h, *e*,  $\tau=144$  h, *f*,  $\tau=168$  h.

The latter molecule has a star shape comprising four aliphatic arms surrounding a central silicon atom. Both compounds are chemically inert.

The liquid drop profiles are shown in Figs 1 and 2. Note the extreme distortion of the horizontal to vertical scales (a factor of  $10^7$ ). The volumes of the drops were found to be conserved (within errors) over the timescale of the experiment (one week), by numerically integrating the profile in cylindrical geometry. This shows that under constant flow of nitrogen there is no evaporation of the liquids.

The first series of profiles (Fig. 1a-c) shows the time evolution of a drop of PDMS. The profiles are extremely flat (with slopes of the order of a few mm per  $10^3$  m). A 'foot', corresponding to the precursor film, is clearly seen extending out of the central cap, (Fig. 1a), with a thickness of 6 Å. This structure may be interpreted as a monolayer of molecules extending for macroscopic distances out from the central macroscopic droplet; the thickness of the 'foot' is comparable to the lateral thickness of the molecule (7 Å) as measured with an atomic force machine (AFM)<sup>13</sup>. As the profile evolves in time (Fig. 1a-c), further structure is visible, with the suggestion of a second layer becoming apparent.

The second series of profiles, for the time evolution of a drop of tetrakis(2-ethylhexoxy)-silane, is more striking. Initially (Fig. 2a) a molecular foot (of thickness 10 Å) extends out from the central reservoir. As the drop spreads and flattens, a second molecular 'step' (again of thickness 10 Å) appears (Fig. 2b), along with some indication of further layers. In Fig. 2c-f the drop flattens down to a 'molecular carpet', and in the transient stages more steps of equal thickness become clearly visible: the first layer continues to extend, at the expense of the upper layers which progressively shrink and disappear. In these transient spreading stages, the fluid is strongly structured, with up to four distinct molecular layers forming near the solid wall. One could consider this layering as a form of smectic-like order induced in the liquid by the presence of an impenetrable wall. We note that the roughness of the substrate must be small for such molecular layering to be possible.

There could be a connection between our observations and adsorption studies of rare gases on graphite, where stepwise adsorption has been observed (ref. 14 and references therein). But it should be emphasized that the layering previously observed in these adsorption experiments corresponds only to equilibrium situations. In our case, the situation is a dynamical one: the layering could occur because of a stacking of the molecules near a wall (smectic density wave<sup>15</sup>) or because of anomalies in the shear viscosity as a function of thickness of the fluid (see, for example, the modified AFM shear-viscosity measurements in ref. 16, which show step-like behaviour).

The dynamic structural effect reported here calls for theoretical efforts aimed at a better understanding of such a striking physical phenomenon. □

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## Greater global warming revealed by satellite-derived sea-surface-temperature trends

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INVESTIGATING the response of global climate to changes in external forcing is essential to our understanding of climate change. Here I present an analysis of satellite-derived sea surface temperatures for the period 1982-88. It can be seen from this analysis that the global ocean is undergoing a gradual but significant warming of  $\sim 0.1^\circ\text{C}$  per year, whereas the trend obtained for the same period from conventional data sources (ships and buoys) is about half that magnitude<sup>1</sup>. Satellite global coverage, however, is far greater and, although we have no long time series of satellite data (as opposed to conventional data), it is possible to observe short-term trends, as shown here, that may not be discerned using the coarser-resolution conventional data.

Monthly mean daytime and night-time multichannel sea-surface-temperature (MCSST) data from NOAA satellites have been consolidated into  $2\frac{1}{2}^\circ$  latitude-longitude quadrangles between  $60^\circ\text{N}$  and  $60^\circ\text{S}$ . Typically, more than two million MCSST determinations are accumulated each month over this area. During the 1982-1988 MCSST record, approximately 98% of this oceanic grid contains MCSST average-temperature determinations over the period.

Using data from the operational Advanced Very-High-Resolution Radiometer (AVHRR), NOAA has developed the capability to measure remotely the surface temperature of the global ocean employing algorithms that effectively correct for attenuation by the intervening atmosphere, which before 1982 had hampered accurate remote measurement of sea surface temperature<sup>2</sup>. Furthermore, these algorithms are initially tuned using measurements of sea surface temperature (SST) from drifting buoys whenever a new satellite is launched, thus assuring continuity of the program between successive sensors<sup>3</sup>. The AVHRR MCSST time series has been carefully monitored on a monthly basis, with SST measurements from drifting buoys being noted so as to guard against instrumental offsets or trends. No significant adjustments have been made to the operational algorithms between different launches of the satellite.

Monthly statistics derived from close individual match-ups between MCSSTs and the *in situ* drifting-buoy data which NOAA receives via the Global Telecommunications System (GTS) have monthly global average biases (both daytime and night-time) within  $\pm 0.3^\circ\text{C}$  (ref. 3). Most (90%) of these biases fall within  $\pm 0.1^\circ\text{C}$ , and match-up scatter (standard deviation of the differences) averages about  $0.6^\circ\text{C}$  (the range is  $0.4$ - $0.9^\circ\text{C}$ ). The buoy-satellite match-up statistics typically represent 300-800 sample pairs per month, both daytime and night-time.

Most significantly in relation to this study, no trends have been observed in these monthly biases and no corrections are made to the operational algorithms on the strength of these biases. Furthermore, no significant biases are observed between daytime and night-time comparisons with drifting buoys, ruling out significant daytime solar heating or 'surface-skin' effects on the monthly means. Because of the paucity of drifting buoys in the Northern Hemisphere that are linked to the GTS, match-ups have been largely restricted to the Southern Hemisphere. A regional survey of bias variability, performed in 1985, revealed no statistically significant variation as a function of atmospheric moisture or location over all major oceanic regions<sup>4</sup>.

A monthly mean consolidation and presentation of MCSST

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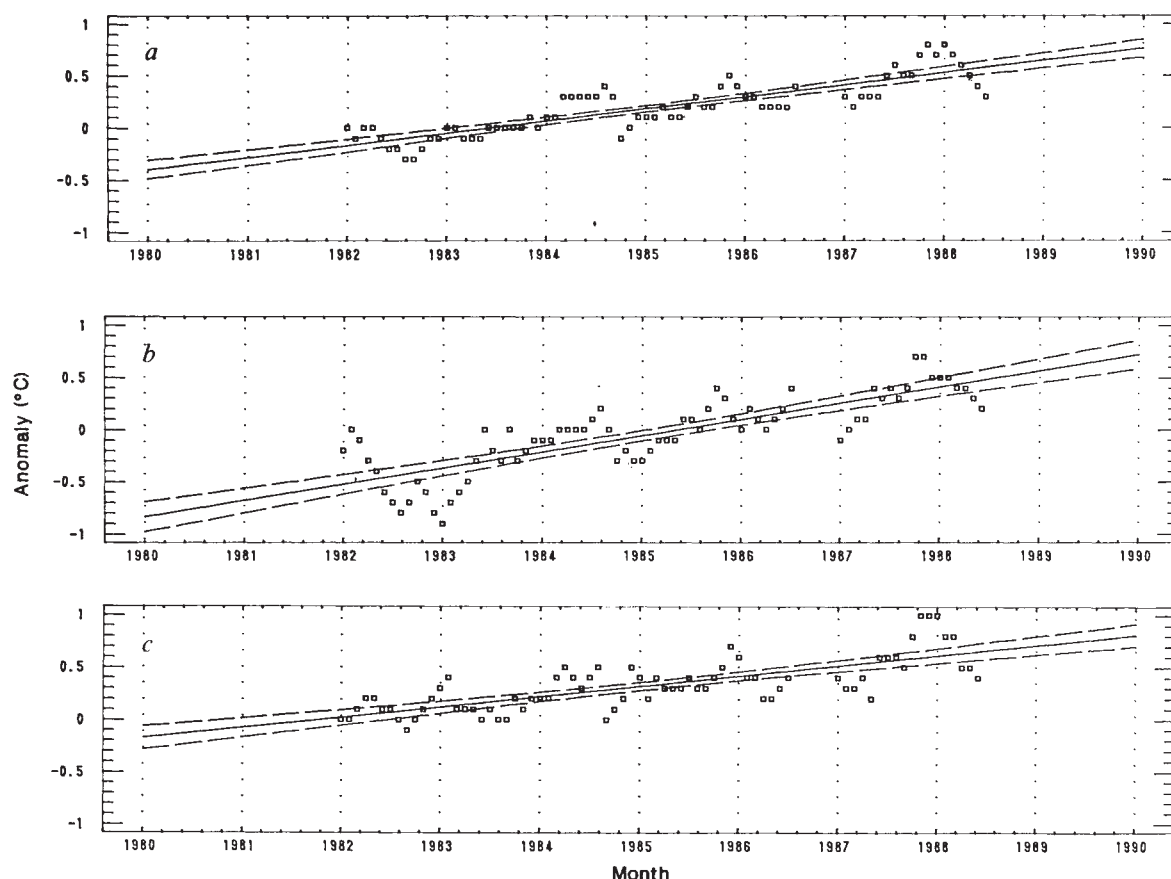


FIG. 1 Satellite-derive multichannel sea-surface-temperature (MCSST) monthly mean departures from climatology ( $^{\circ}\text{C}$ ) for the period 1982 to June 1988. *a*, Global: slope  $b=0.12\text{ }^{\circ}\text{C yr}^{-1}$ , variance  $r^2=0.73$ ; *b*, Northern Hemisphere (NH):  $b=0.16\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.66$ ; *c*, Southern Hemisphere (SH):  $b=0.1\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.57$ . Regression lines include their confidence limits at

95% level (dashed lines). (With the El Chichon years of 1982 and 1983 excluded (see text) the slope and variance values shift slightly: global— $b=0.11\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.46$ ; NH— $b=0.13\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.53$ ; SH— $b=0.1\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.33$ .)

TABLE 1 Monthly variations in mean SST

| Month     | Slope (all months)    |                     |                     | Slope (1982 and 1983 excluded)    |                     |                     |
|-----------|-----------------------|---------------------|---------------------|-----------------------------------|---------------------|---------------------|
|           | Global                | Northern Hemisphere | Southern Hemisphere | Global                            | Northern Hemisphere | Southern Hemisphere |
| January   | 0.11                  | 0.14                | 0.13                | 0.11                              | 0.09                | 0.14                |
| February  | 0.11                  | 0.11                | 0.08                | 0.11                              | 0.07                | 0.11                |
| March     | 0.09                  | 0.11                | 0.09                | 0.08                              | 0.07                | 0.08                |
| April     | 0.08                  | 0.12                | 0.04                | 0.06                              | 0.01                | 0.01                |
| May       | 0.08                  | 0.13                | 0.03                | 0.04                              | 0.11                | 0.01                |
| June      | 0.08                  | 0.11                | 0.08                | 0.03                              | 0.06                | 0.05                |
| July      | 0.15                  | 0.21                | 0.10                | 0.10                              | 0.12                | 0.06                |
| August    | 0.14                  | 0.20                | 0.12                | 0.05                              | 0.05                | 0.05                |
| September | 0.14                  | 0.19                | 0.12                | 0.11                              | 0.11                | 0.14                |
| October   | 0.18                  | 0.26                | 0.16                | 0.20                              | 0.28                | 0.17                |
| November  | 0.18                  | 0.26                | 0.19                | 0.20                              | 0.24                | 0.23                |
| December  | 0.16                  | 0.23                | 0.17                | 0.18                              | 0.18                | 0.19                |
| Month     | Variance (all months) |                     |                     | Variance (1982 and 1983 excluded) |                     |                     |
|           | Global                | Northern Hemisphere | Southern Hemisphere | Global                            | Northern Hemisphere | Southern Hemisphere |
| January   | 0.77                  | 0.50                | 0.76                | 0.74                              | 0.46                | 0.80                |
| February  | 0.79                  | 0.45                | 0.55                | 0.77                              | 0.38                | 0.77                |
| March     | 0.72                  | 0.58                | 0.66                | 0.69                              | 0.69                | 0.59                |
| April     | 0.70                  | 0.78                | 0.35                | 0.41                              | 0.68                | 0.01                |
| May       | 0.73                  | 0.89                | 0.25                | 0.31                              | 0.70                | 0.01                |
| June      | 0.67                  | 0.68                | 0.69                | 0.15                              | 0.69                | 0.37                |
| July      | 0.93                  | 0.88                | 0.85                | 0.83                              | 0.80                | 0.60                |
| August    | 0.75                  | 0.73                | 0.73                | 0.25                              | 0.25                | 0.25                |
| September | 0.85                  | 0.78                | 0.93                | 0.79                              | 0.94                | 0.91                |
| October   | 0.89                  | 0.90                | 0.89                | 0.85                              | 0.88                | 0.86                |
| November  | 0.89                  | 0.96                | 0.91                | 0.85                              | 0.93                | 0.98                |
| December  | 0.97                  | 0.85                | 0.96                | 0.98                              | 0.78                | 0.97                |



measurements has been available for global evaluation since 1982<sup>5</sup>. This study presents an analysis of MCSSTs for a 6½-year time series beginning in 1982 and extending to mid-1988. On a 2½° latitude-longitude grid, the Earth's oceans occupy ~5,000 of these grid boxes. An average of ~500 monthly MCSST observations accumulate at each grid location. An exception to the otherwise nearly perfect coverage in this ±60° zonal band occurred during 1982 and into the first half of 1983, when monthly mean accumulations became briefly less dense, particularly in the Northern Hemisphere tropics. This was a result of contaminating aerosol from El Chichón precluding MCSST retrievals through the thickest portions of the extensive volcanic cloud<sup>6</sup>.

To provide for further evaluation of the MCSST data, 2½° latitude zonal means are calculated; these are then used to compute MCSST means for the Northern and Southern Hemispheres. Before values for each hemisphere are composited, zonal means are appropriately weighted to allow for the varying number of oceanic grid points in each zonal band. A similar treatment is applied to the global ensemble of MCSST data to produce an integrated monthly mean value for the entire ocean data set. An alternative evaluation, which weights grid points by area, produces similar results.

One small segment of the time series is not available at present. This 5-month lapse occurred in 1986 between August and December because of problems with the computer system. These data cannot be used until arrangements for reprocessing can be made.

Global, hemispheric and selected ocean basins were examined for significant MCSST trends during the 1982–1988 period using simple regression techniques. Departures from Robinson–Bauer climatology<sup>7,8</sup> (that is, anomalies) are used in all cases to normalize results. Global MCSST tendencies, with statistics, are shown in Fig. 1 with 95% confidence limits for the slope. Standard

errors of all slopes depicted in Fig. 1 never exceeded  $\pm 0.01$  °C yr<sup>-1</sup>. In addition, a separate analysis was performed in which all 1982 and 1983 data were excluded from the 6½-year time series to reduce potential effects of stratospheric aerosol contamination from El Chichón. These small differences are noted in the legend for Fig. 1.

Another analysis was performed to investigate trends of the monthly data sets by calendar month. This allows us to focus on any exceptional tendencies during certain months or seasons. The data from Fig. 1 are redistributed for this purpose and are shown in Fig. 2 and tabulated in Table 1.

Figure 3 presents trends of the world's oceans subdivided into five major regions: North and South Atlantic; North and South Pacific; and Indian. Standard errors of the slopes in Fig. 3 ranged from  $\pm 0.01$  to  $0.02$  °C yr<sup>-1</sup>.

Comparisons with past time series are difficult as no truly complete data sets were derived from solely conventional data, for which considerable smoothing is required to produce acceptable global analyses. An analysis of historical SST data by Oort *et al.*<sup>9</sup> also revealed an increased rate of temperature increase in the Northern relative to the Southern Hemisphere, but with a magnitude only half that of the total increase found here for the 1980s. Furthermore, the gradual increase presented in the Oort *et al.* survey occurred over a fifty-year period (1910–1960).

A recent study<sup>1</sup> incorporating data from a Comprehensive Ocean–Atmosphere Data Set (COADS) has also shown increasing surface temperatures (land and sea) during this same time interval, but the observed trend is only about half that found here. Even though some ocean surface temperatures were included, no satellite-derived sea surface temperatures were used in the COADS data sets<sup>10</sup>. Trends in the COADS data may be suppressed because ship and buoy observations typically miss over 25% of the monthly 2½° grid points south of 12.5°N, where many monthly means must be calculated from fewer than five

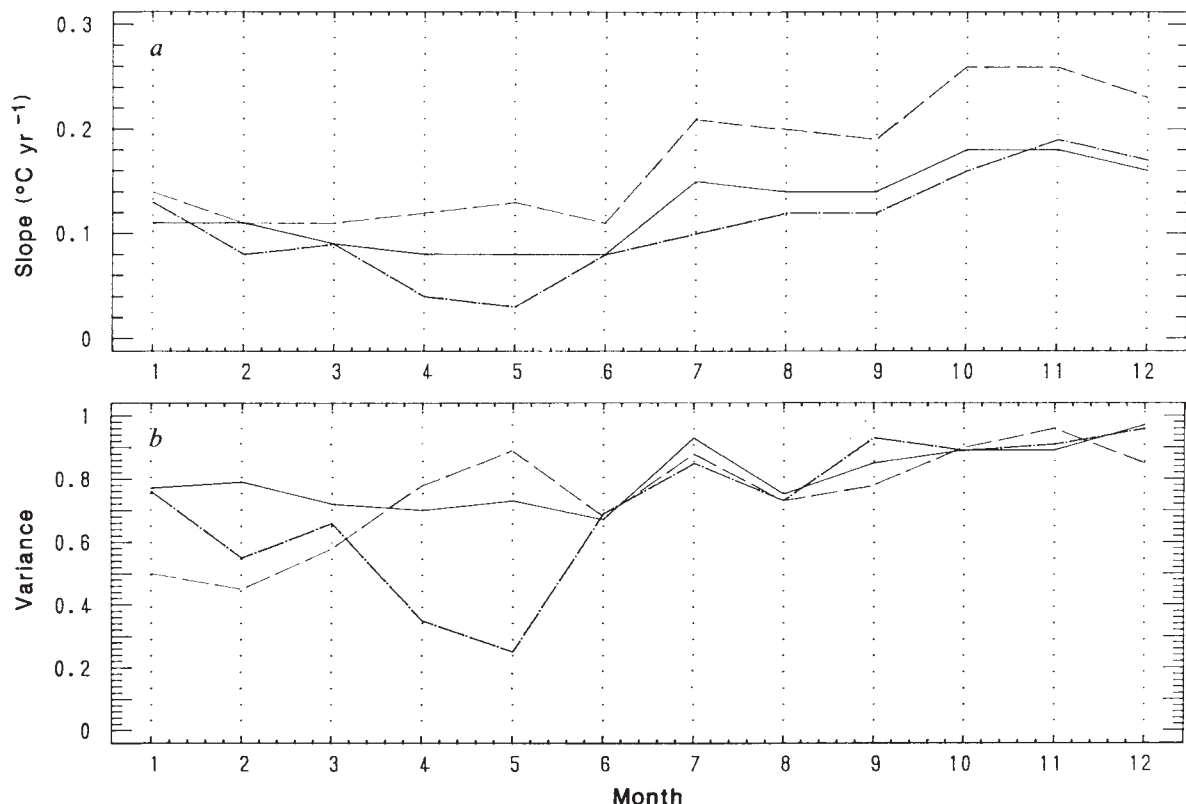


FIG. 2 MCSST monthly mean tendencies by month for global (solid line), Northern (dashed line) and Southern (dot-dashed line) Hemispheres. a,

Regression slopes (°C yr<sup>-1</sup>). b, Variances ( $r^2$ ).

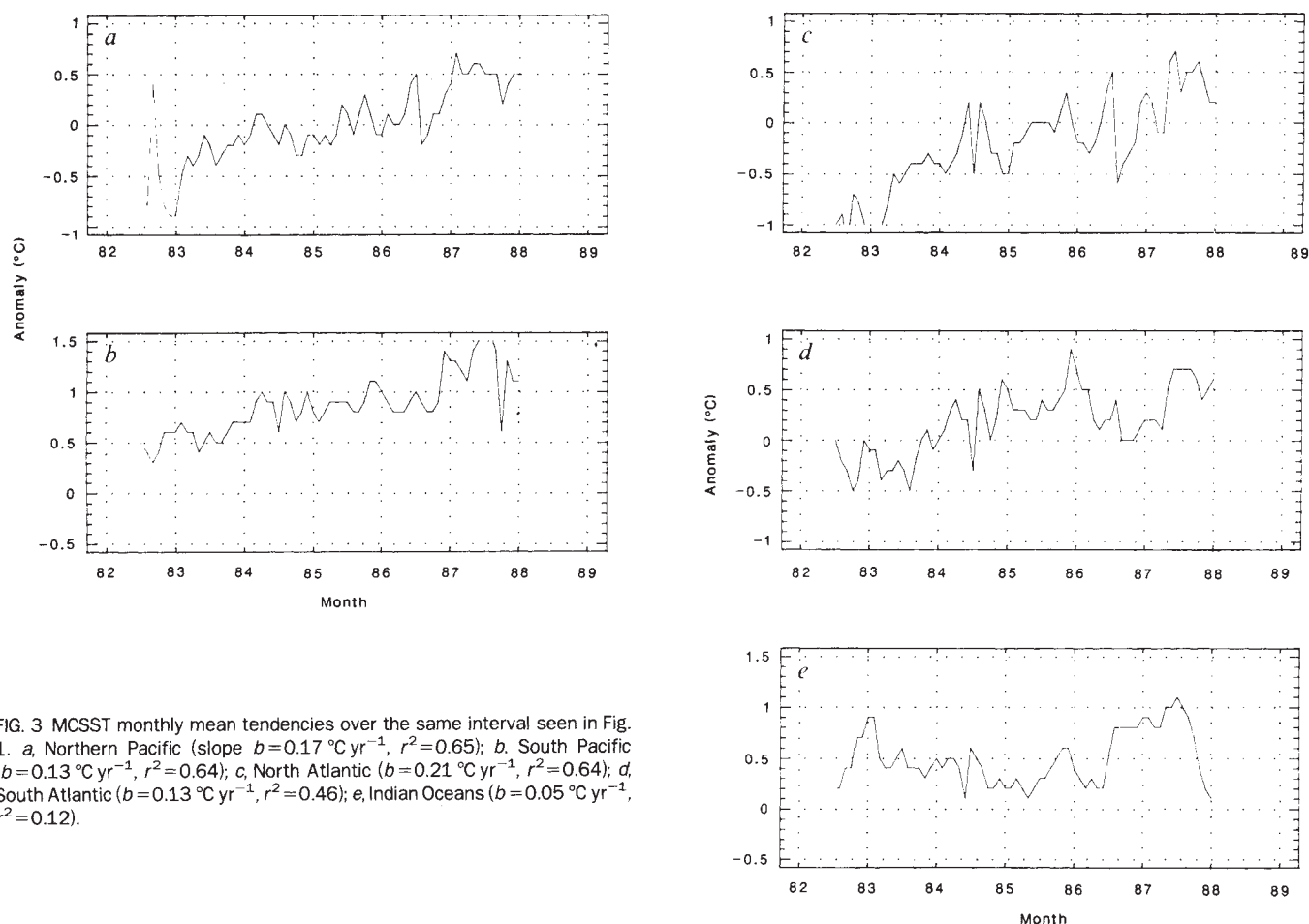


FIG. 3 MCSST monthly mean tendencies over the same interval seen in Fig. 1. *a*, Northern Pacific (slope  $b=0.17\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.65$ ); *b*, South Pacific ( $b=0.13\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.64$ ); *c*, North Atlantic ( $b=0.21\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.64$ ); *d*, South Atlantic ( $b=0.13\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.46$ ); *e*, Indian Oceans ( $b=0.05\text{ }^{\circ}\text{C yr}^{-1}$ ,  $r^2=0.12$ ).

observations. COADS data coverage typically exceeds 90% only at grid points between  $20^{\circ}$  and  $40^{\circ}$  N. Furthermore it is possible that the land temperature, whose contribution to the total area is nearly twice as great in the Northern than in the Southern Hemisphere, may be warming at a different (slower?) rate than the ocean.

Further inspection of specific regions reveals increasing MCSST tendencies that are similar in the Atlantic and Pacific basins but are insignificant over the Indian Ocean. Closer examination of zonal tendencies, or even pinpointing the 'centres of action' for the months or zonal bands exhibiting marked warming, would seem to be warranted.

When examined on a separate monthly basis, the months of October to December are characterized by considerably more significant MCSST increases than March to June in both hemispheres (Fig. 2). Although this is undoubtedly partly a result of the 1987 El Niño, October and November rates exceed  $0.2\text{ }^{\circ}\text{C yr}^{-1}$  in the Northern Hemisphere, with explained variances consistently exceeding 0.90.

With the collection of ship data from thousands of reports having been necessary each month over the last dozen decades<sup>9</sup>, the incorporation of satellite-derived, buoy-maintained SSTs would seem to have certain advantages. Using the generally high-quality drifting-buoy data alone to corroborate observed trends in the global MCSST data set would not appear practical because of their paucity and spatial variability from year to year. Nevertheless, these drifters are an invaluable part of the satellite MCSST program. Unfortunately, no other adequate

independent system exists for rigorous validation. Long time-series data from moored buoys could be helpful for further comparisons at key sites. But where should these sites be located? Studies are underway to better identify regions showing the greatest trends in MCSST, with the hope that larger sets of quality-controlled conventional data can be secured in these more limited areas.

Global changes of the sort described here have been predicted to increase dramatically over the next few decades as a result of the greenhouse effect. We may be just beginning to witness the onset of this warming through satellite surveillance of ocean surface temperature. Using satellite MCSST time-series data, we are now in a better position to monitor both long- and short-term oceanic changes that, before this decade, were largely unnoticed. □

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# The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity

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THE development of the body plan in the *Drosophila* embryo depends on the activity of maternal determinants localized at the anterior and posterior of the egg. These activities define both the polarity of the anterior-posterior (AP) axis and the spatial domains of expression of the zygotic gap genes<sup>1,2</sup>, which in turn control the subsequent steps in segmentation<sup>2,3</sup>. The nature and mode of action of one anterior determinant, the *bicoid* (*bcd*) gene product, has recently been defined<sup>4-8</sup>, but the posterior determinants are less well characterized. At least seven maternally acting genes are required for posterior development<sup>1,9-11</sup>. Mutations in these maternal posterior-group genes result in embryos lacking all abdominal segments. Cytoplasmic transplantation studies indicate that the maternally encoded product of the *nanos* (*nos*) gene may act as an abdominal determinant, whereas the other maternal posterior-group genes appear to be required for the appropriate localization and stabilization of this signal<sup>1,9-12</sup>. Here we show that the lack of the *nos* gene product can be compensated for by eliminating the maternal activity of the gap gene *hunchback* (*hb*). Embryos lacking both of these maternally derived gene products are viable and can survive as fertile adults. These results suggest that the *nos* gene product functions by repressing the activity of the maternal *hb* products in the posterior of the egg.

The *hunchback* gene is the zygotic gap gene required for the formation of segments in the head and anterior thorax<sup>13</sup>. Localized transcription of *hb* in the anterior half of the embryo is induced at late cleavage stages by a gradient of *bcd* protein<sup>14,15</sup>,

and the resulting zygotically derived *hb* protein can be detected by the eleventh nuclear division<sup>14</sup>. In addition, there is also a maternal contribution to *hb* activity in the early embryo. Maternal *hb* transcripts are uniformly distributed throughout the egg at the time of fertilization, but by the eighth nuclear division they are present in an anterior to posterior gradient; at approximately the same time *hb* maternal protein can be detected in a similar gradient<sup>14,16</sup>. This maternally derived *hb* protein is not required for normal development, because embryos derived from homozygous mutant mothers can be rescued by a paternally derived wild-type copy of the gene<sup>13</sup>. But it is functional, for elimination of both maternal and zygotic *hb* expression results in a stronger mutant phenotype than elimination of only the zygotic expression (ref. 13; see Fig. 1). The maternal and zygotic *hb* transcripts arise from different promoters, but both appear to encode the same product, a protein which contains a DNA-binding zinc-finger domain<sup>16</sup>.

Mutations in the maternal posterior-group genes *nos*, *oskar*, *vasa* and *staufer* alter the graded distribution of *hb* maternal protein; these mutations all lead to the accumulation of maternal *hb* protein at a uniform, relatively high level throughout the entire embryo<sup>14</sup>. This alteration has little effect on the *bcd* dependent zygotic activation of *hb* transcription<sup>14</sup>. However, the effects of *oskar* mutations on the transcription of homeotic genes in the blastoderm<sup>17</sup> led us to suspect that the phenotype of these posterior-group mutants may result, at least in part, from the presence of *hb* protein in the posterior of the embryo.

To test whether the effect of the maternal posterior-group genes was mediated primarily by the maternal *hb* gene product, we examined the phenotype of embryos lacking the maternally derived products of both the *hb* and *nos* genes (Figs 1 and 2). To generate these embryos, we constructed chimaeric mothers carrying homozygous *hb<sup>-</sup>nos<sup>-</sup>* germ-line tissue (see Table 1 for details of these pole cell transplantation experiments). Individual chimaeric females carrying either *hb<sup>-</sup>nos<sup>-</sup>/++* or *hb<sup>-</sup>nos<sup>-</sup>/hb<sup>-</sup>nos<sup>-</sup>* germ-line tissue were mated to *hb<sup>-</sup>/+* males and the resulting progeny were scored for survival and cuticle phenotype. Progeny deriving from heterozygous germ lines showed the expected ratios of surviving normal embryos and lethal embryos displaying the zygotic *hb<sup>-</sup>* phenotype (Table 2). Progeny deriving from homozygous *hb<sup>-</sup>nos<sup>-</sup>* germ lines fell into two classes. About half of these displayed a phenotype indistinguishable from that of *hb<sup>-</sup>/hb<sup>-</sup>* embryos derived from *hb<sup>-</sup>/hb<sup>-</sup>*

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TABLE 1 Generation of chimaeric mothers by pole cell transplantation

| Crosses to generate donor embryos*                                                                                                                              | Number of transplants† | Number of surviving females | Number of fertile females | Genotype of donor germ-line received by fertile females‡                                                               |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|-----------------------------|---------------------------|------------------------------------------------------------------------------------------------------------------------|
| Experiment 1<br>♀ <i>hb<sup>TM48</sup>/TM1, cu</i> ×<br>♂ <i>hb<sup>TM48</sup>/Df(3R)pXT103; Dp(3:Y)P92</i>                                                     | 340                    | 35                          | 4                         | 3 <i>hb<sup>-</sup>/TM1, cu</i><br>1 <i>hb<sup>-</sup>/hb<sup>-</sup></i>                                              |
| Experiment 2<br>♀ <i>hb<sup>TM48</sup> nos<sup>L7</sup>/TM1, cu</i> ×<br>♂ <i>hb<sup>TM48</sup> nos<sup>L7</sup>/Df(3R)pXT103, nos<sup>L7</sup>; Dp(3:Y)P92</i> | 947                    | 148                         | 13                        | 7 <i>hb<sup>-</sup>nos<sup>-</sup>/TM1, cu</i><br>6 <i>hb<sup>-</sup>nos<sup>-</sup>/hb<sup>-</sup>nos<sup>-</sup></i> |
| Experiment 3<br>♀ <i>nos<sup>L7</sup>/TM3</i> ×<br>♂ <i>nos<sup>L7</sup>/nos<sup>L7</sup></i>                                                                   | 223                    | 37                          | 3                         | 2 <i>nos<sup>-</sup>/TM3</i><br>1 <i>nos<sup>-</sup>/nos<sup>-</sup></i>                                               |

\* *Df(3R)pXT103* is a deletion removing the chromosomal region containing *hb* (ref. 13). *Dp(3:Y)P92* contains a functional copy of the *hb* gene expressing the Rg(pbx) phenotype<sup>13</sup>. This duplication allows for homozygosity of *hb<sup>-</sup>* on the autosomes. Half of the resulting donor females will be null for *hb* (ref. 13).

† Pole cell transplants were performed as described in ref. 13. Pole cells from donor embryos were transplanted into recipient embryos derived from a cross of *+/+* females (Canton-S stock) to *OvoD1/Y* males. *OvoD1* is a germ-line autonomous dominant female sterile mutation. All females resulting from this cross are sterile and lay no eggs. Those receiving female donor pole cells will have a mosaic germ line composed of both donor and recipient cells and will produce eggs only from the donor tissue.

‡ The genotype of each germ line was determined by crossing mosaic females with *hb<sup>TM48</sup>/TM3* males. All mosaic females classified as *hb<sup>-</sup>/+* or *hb<sup>-</sup>nos<sup>-</sup>/+* on the basis of cuticle phenotypes transmitted the balancer chromosome *TM1* to their progeny. No females similarly classified as carrying *hb<sup>-</sup>/hb<sup>-</sup>* or *hb<sup>-</sup>nos<sup>-</sup>/hb<sup>-</sup>nos<sup>-</sup>* germ lines transmitted the balancer. Three of the six *hb<sup>-</sup>nos<sup>-</sup>* homozygous germ lines transmitted both the *TM48* and the *Df(3R)pXT103* chromosomes (distinguished by the *pink* allele uncovered by *Df(3R)pXT103*). One such *hb<sup>-</sup>nos<sup>-</sup>/hb<sup>-</sup>nos<sup>-</sup>* germ line was further tested for the presence of *nos<sup>-</sup>* on both homologues. Of 18 chromosomes tested, all gave sterile females laying typical *hb<sup>-</sup>nos<sup>-</sup>/nos<sup>-</sup>* eggs when crossed to *nos<sup>L7</sup>* males.



germ lines (Fig. 1). We assume that these lethal embryos are genotypically  $hb^{-nos^-}/hb^{-}$ . The remaining sibling embryos appeared normal and many survived to adulthood. We confirmed that this viable class of progeny carried the maternally derived  $hb^{-nos^-}$  chromosome by subsequent genetic analysis (Table 1). Although these viable progeny were derived from a homozygous  $nos^-$  germ line, the absence of the  $hb$  maternal component completely compensated for the normally lethal  $nos$  maternal effect. Hülkamp *et al.* have obtained similar results in analogous pole cell transplantation experiments using another allele of  $hb$  (see accompanying article<sup>22</sup>).

The rescue of the lethal  $nos$  maternal effect is not due to the influence of somatically produced  $nos$  gene product in the mother: chimaeric mothers that carry a  $nos^-/nos^-$  mutant germ-

line but wild-type somatic tissue produce embryos that show a typical  $nos$  mutant phenotype (Fig. 2b).

These results indicate that the only essential function of the  $nos$  gene product is to regulate the activity of the  $hb$  maternal gene product. This regulation probably occurs at the level of translational control, with  $nos$  acting to repress efficient translation of the maternal  $hb$  RNA in the posterior of the egg<sup>14</sup>.

The role of  $nos$  as a repressor of maternal  $hb$  activity differs markedly from the previously proposed function of the maternal posterior-group genes. The anterior domain of zygotic  $hb$  expression is under the positive control of the  $bcd$  gene product<sup>14,15</sup>. By analogy, the maternal posterior-group genes have been assumed to activate the zygotic gap gene *knirps* (*kni*) in the posterior of the embryo (for reviews, see refs 1-3). Although

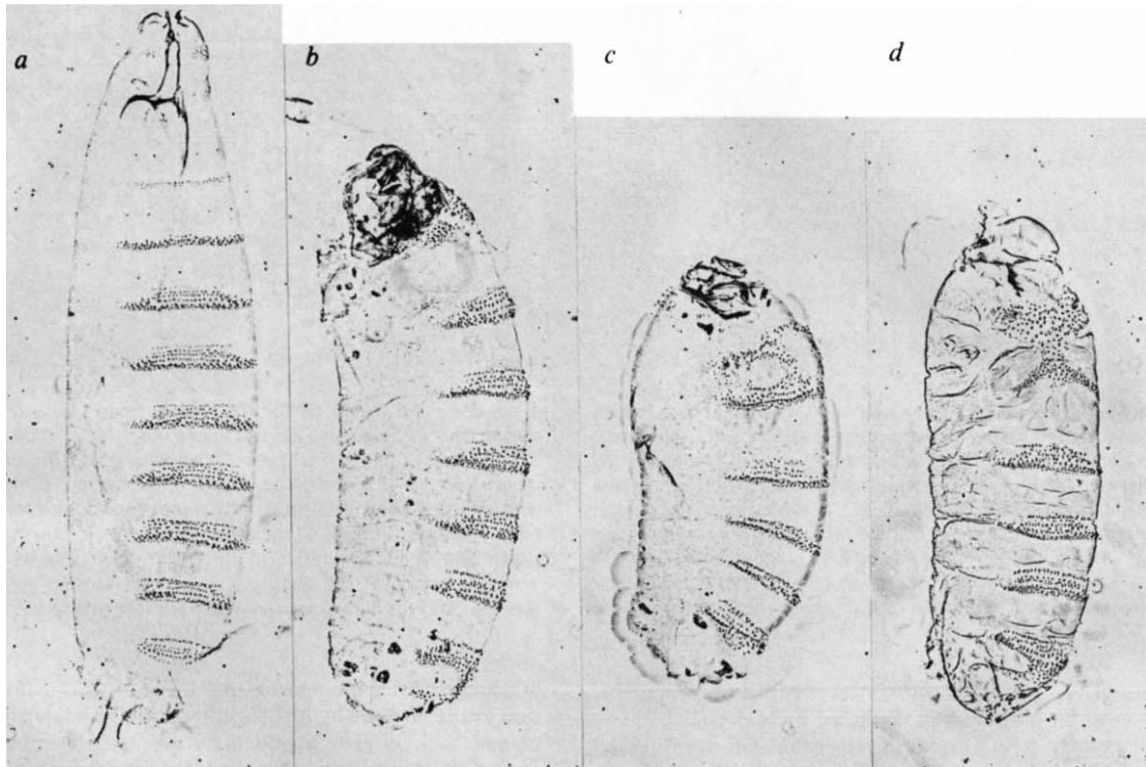


FIG. 1 Cuticle preparations of embryos mutant for various combinations of  $nos$  and  $hb$  maternal and zygotic gene products. *a*, Wild-type. For a description of the wild-type pattern, see ref. 21. *b*, Embryo hemizygous for  $hb^{TM48}$ . This  $hb$  allele behaves as an *amorph*<sup>9</sup>, and produces no detectable protein<sup>8</sup>. Parts of the labial and all the thoracic segments are missing, and abdominal segments A7 and A8 are fused. *c*, Embryo lacking both maternal and zygotic  $hb$  gene products (see Table 1, experiment 1, for a description of the generation of these embryos). Anteriorly, two abdominal segments of reversed polarity are formed. Posteriorly, abdominal segments A3-6 and

the fused A7/8 are formed with normal polarity. The location of the plane of symmetry is somewhat variable<sup>13</sup>; in this embryo, it runs through anterior A3. *d*, Embryo lacking maternal  $nos$  and both maternal and zygotic  $hb$  gene products (see Table 1, experiment 2, for the generation of this class of embryos). This embryo displays a phenotype similar to that of the embryo in *c*. The spectrum of phenotypes produced in this experiment is indistinguishable from that of embryos produced in experiment 1. Phase contrast photographs of cuticle preparations as in ref. 13.

TABLE 2 Progeny of  $hb^{-nos^-}$  homozygous and heterozygous germ lines

| Cross generating embryos                                         | Total fertilized eggs | Dead $hb^{-}$ phenotype embryos | Wild-type |         |         | Unclassified embryos (died early or lost) | Unfertilized eggs |
|------------------------------------------------------------------|-----------------------|---------------------------------|-----------|---------|---------|-------------------------------------------|-------------------|
|                                                                  |                       |                                 | Total     | Hatched | Eclosed |                                           |                   |
| ♀ Germ line: $hb^{-nos^-}/hb^{-nos^-}$<br>♂ Genotype: $hb^{-}/+$ | 108                   | 49                              | 48        | 42      | 22      | 11                                        | 50                |
| ♀ Germ line: $hb^{-nos^-}/+$<br>♂ Genotype: $hb^{-}/+$           | 167                   | 40                              | 113       | 102     | 75      | 14                                        | 23                |

Counted eggs from fertile transplant females of each genotype were allowed to develop for 24-48 h. Unhatched eggs were removed, dechorionated and classified as unfertilized eggs, dead embryos arrested before cuticle formation, or embryos arrested after cuticle formation. The latter were classified as wild-type or hunchback cuticle patterns. Hatching larvae (assumed in the table to be wild-type) were allowed to develop to adults. The emerging adults were normal fertile flies with no obvious segmentation defects. A separate sample of eggs were sacrificed at around the time of hatching. All cuticularised embryos showed either a hunchback phenotype, or a complete array of denticle belts. Of 18 non-hunchback embryos, 12 showed completely normal segmentation, whereas six had minor defects in individual denticle belts.

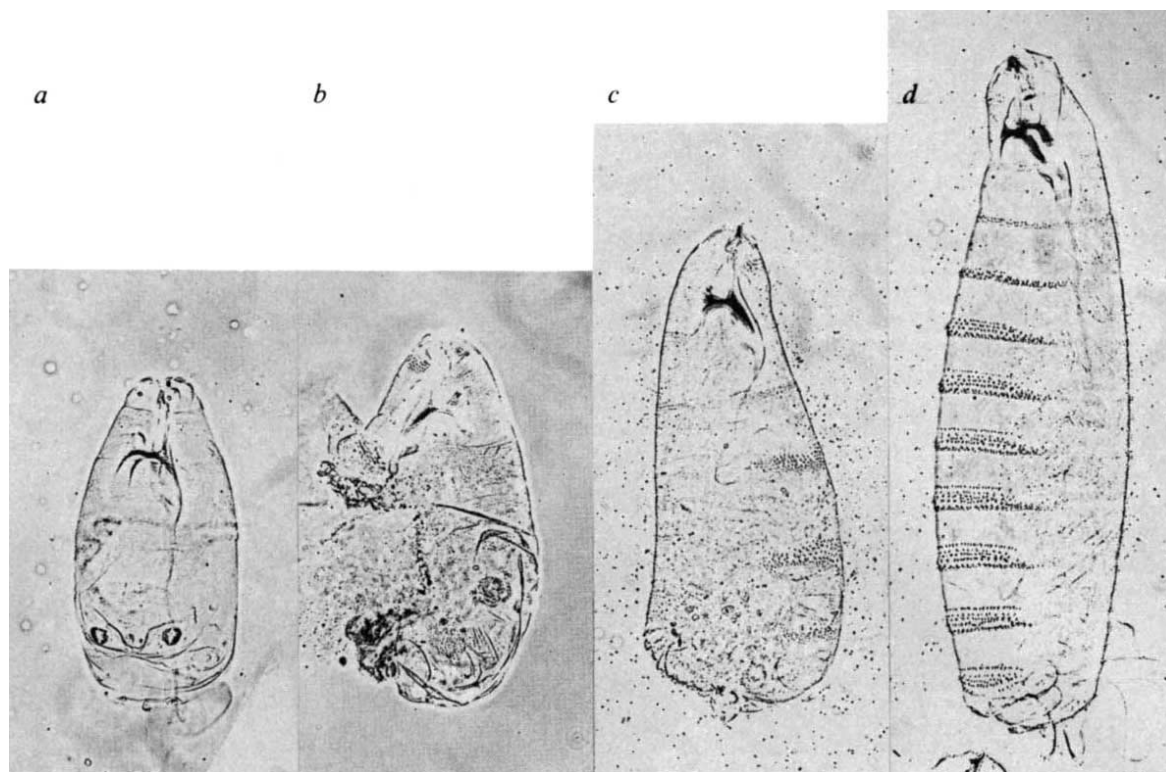


FIG. 2 Phenotypes of *nos* embryos with decreasing dosage of maternal *hb* genes. *a*, Embryo derived from a mother homozygous for *nos*<sup>L7</sup>, with normal genomic complement of *hb*<sup>+</sup>. Head, thorax and telson are normal, but all abdominal segments are missing. Occasionally in this genotype, a small patch of abdominal denticles is located in the otherwise naked region between the third thoracic segment and the feizkörper. *b*, Embryo derived from a *nos*<sup>L7</sup> homozygous germ-line (see Table 1, experiment 3 for the generation of such embryos). The *nos* phenotype is unaffected by the wild-type somatic tissue of the mother. This embryo is distorted by rupture

in the abdominal region. Similar ruptures commonly occur in the cuticle of all *nos* embryos. *c*, Embryo derived from a *nos*<sup>L7</sup> *hb*<sup>TM48</sup>/*nos*<sup>L7</sup> mother, with only one dose of *hb*<sup>+</sup>. The presence of two abdominal denticle belts indicates a partial rescue of abdominal phenotype. This rescue is somewhat variable, with embryos having on average between one and three abdominal denticle belts. *d*, Embryo lacking all maternal *hb* and *nos* products (see Table 1, experiment 2, for generation of these embryos). This embryo shows a complete rescue of the abdominal phenotype, with no detectable abnormalities. Embryos of this class are viable and fertile. Preparations as in Fig. 1.

*nos* is required for *kni* expression<sup>18</sup>, our result imply that this interaction must be indirect and mediated by maternal *hb* gene products. Presumably, in normal development *kni* is expressed only where it is not repressed by the *hb* gene product. This is consistent with the phenotype of embryos lacking all *hb* gene products (Fig. 1*c, d*). In these animals, abdominal segments are formed both anteriorly and posteriorly, suggesting that *kni* is ectopically expressed when the *hb* gene products are absent. Mutations in *nos* must also have indirect effects on the expression of genes other than *kni*, because the phenotype of embryos lacking the *nos* gene product is different from that of *kni*<sup>-</sup> embryos<sup>19,20</sup>. It is likely that repression of other genes by maternal *hb* protein accounts for this difference.

The degree of abdominal development is sensitive to the level of maternal *hb* expression. By varying the dosage of maternal *hb* genes, it is possible to titrate the effect of mutations in the maternal posterior-group genes (Fig. 2). The abdominal phenotype produced by mutations in *nos* (Fig. 2*a* and *b*) is partially rescued by eliminating one copy of the maternal *hb* gene (Fig. 2*c*). These embryos develop one to three abdominal denticle belts. The complete lack of maternal *hb* expression results in full restoration of the abdomen (Fig. 2*d*). Conversely, ectopic overexpression of the *hb* gene product can result in an embryonic lethal phenotype that resembles that produced by mutations in the posterior group genes (Hülskamp *et al.*, this issue<sup>22</sup>; G. Struhl, manuscript submitted).

There is no obvious reason why the maternal *hb* and *nos* gene products should have been conserved over time, because the elimination of both by mutation results in a viable fly. It may be that we are seeing in *Drosophila* an evolutionary relic of a

mechanism that was once essential. The localized anterior activation of the *hb* gene by *bcd*, together with the posterior repression of maternal *hb* gene products by *nos*, may be relatively recent evolutionary acquisitions, associated with the simultaneous formation of anterior and posterior segments in long germ-band insects. In short germ-band insects, where only the anterior segments are defined initially, the maternal *hb* gene product may itself act as an anterior determinant. □

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# The B-cell binding site on human immunoglobulin E

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**IMMUNOGLOBULIN E** comprises the main immunoglobulin class associated with allergy. Its multifarious activities are mediated by two types of Fc receptors found on different cell populations, FcεR1 on mast cells and basophils<sup>1</sup>, and FcεR2 on inflammatory cells (monocytes, eosinophils and platelets<sup>2</sup>) and B lymphocytes<sup>3</sup>. Recombinant ε-chain fragments synthesized in *Escherichia coli* have provided the means of mapping the receptor-binding sites on human IgE, and blocking IgE-receptor interactions. We have previously shown that the FcεR1 binding site is contained within a sequence (Gln 301–Arg 376) spanning the Cε2 and Cε3 domains<sup>4</sup>. Here we show that FcεR2 can recognize a motif in the Cε3 domain that is formed on dimerization of one or both of the flanking (Cε2 and Cε4) domains. Glycosylation of IgE is not required for the activity of either receptor.

Table 1 and Figure 1 describe the recombinant ε-chain (rE) peptides used in this study. Like the myeloma protein IgE(PS), rE2–4, rE2'–4 and rE3–4 bound to ≥90% of FcεR2-positive RPMI 8866 B cells, as assessed by indirect immunofluorescence. By contrast, there was no detectable binding of rE2–3, rE2'–3', rE4 and rE2. The binding of the rE peptides was specific, in that it could be inhibited by preincubation of the cells with two anti-FcεR2 monoclonal antibodies (mAb135 and anti-BLAST-2), but not by control IgG<sub>1</sub> of unrelated specificity (data not shown). Moreover, there was no binding to the FcεR2-negative cell lines Jurkat and Raji (data not shown). The data in Table 1 indicate that the FcεR2 binding site is contained in the rE3–4 peptide (Leu 340–Lys 547); the Cε2 domain is not required.

TABLE 1 Binding of recombinant IgE peptides to RPMI 8866 B cells by indirect immunofluorescence

| Ligand  | Amino-acid sequence | % Positive cells |
|---------|---------------------|------------------|
| IgE(PS) | Glp 1–Lys 547       | 91 ± 2           |
| rE2–4   | Asp 218–Lys 547     | 92 ± 2           |
| rE2'–4  | Gln 301–Lys 547     | 91 ± 2           |
| rE3–4   | Leu 340–Lys 547     | 90 ± 3           |
| rE2–3   | Asp 218–Pro 439     | 3 ± 1            |
| rE2'–3' | Gln 301–Arg 376     | 2 ± 1            |
| rE2     | Asp 218–Val 336     | 2 ± 1            |
| rE4     | Arg 440–Lys 547     | 3 ± 2            |

The rE peptides were previously used to map the IgE binding site for the mast cell receptor and are described fully in earlier work<sup>4</sup>. For indirect immunofluorescence,  $0.5 \times 10^6$  RPMI 8866 cells (>99% FcεR2-positive) in staining buffer (RPMI 1640 containing 2.5% fetal bovine serum and 0.01% azide) were incubated with various concentrations ( $0.1$ – $200 \mu\text{g ml}^{-1}$ ) of purified rE fragments or native IgE(PS) (gift from K. Ishizaka) for 40 min at 4 °C. Similar results were obtained with incubation times of 2 and 3 h. After washing, the cells were incubated for 30 min at 4 °C with the appropriate fluorescein isothiocyanate-conjugated anti-Fcε mAb (see Table 2) or with an affinity-purified goat anti-human IgE antibody ( $10 \mu\text{g ml}^{-1}$ ). After extensive washing, the percentage of cells binding IgE or the rE fragments was evaluated by a FACScan (Becton Dickinson). The table shows the absolute percentage of positive cells (mean ± s.d. of results obtained in 9 experiments).

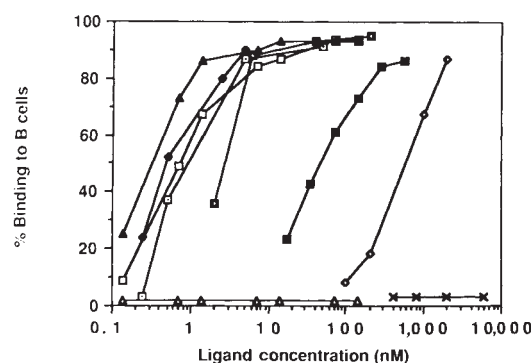


FIG. 1 Binding ability of recombinant ε-chain peptides. IgE, □; deglycosylated IgE, ◆; rE2–4, □; rE2'–4, ■; rE2–3, ×; rE3–4, ◇; dimeric rE2–4 (Arg 506), ▲; monomeric rE2–4 (Arg 506), △; rE2'–3/G3, ■. The concentrations required for 50% binding to the B-cell line were: IgE,  $9.0 \times 10^{-10}$  M; deglycosylated IgE,  $4.8 \times 10^{-10}$  M; rE2–4,  $7.2 \times 10^{-10}$  M; rE2'–4,  $2.6 \times 10^{-9}$  M; rE3–4,  $5.8 \times 10^{-7}$  M; dimeric, rE2–4 (Arg 506),  $1.6 \times 10^{-10}$  M; rE2'–3/G3,  $4.7 \times 10^{-8}$  M.

**METHODS.** The rE peptides were isolated from *E. coli* and affinity-purified as described<sup>4</sup>. The ε/γ chimaeric recombinant was constructed using cDNA sequence from pHG201 (ref. 11). A partial *SacI* digest yielded a fragment encoding residues 346–447 (EU index) plus 3' untranslated sequence, which was shortened by T4 polymerase after insertion in the correct reading frame downstream of Cε3 at an *FnuDII* site. The recombinant peptide reacted with both ε and γ2b antisera. The point substitutions in rE2–4 were made by oligonucleotide-directed site-specific mutagenesis using double-stranded plasmid DNA (ref. 12). For deglycosylation, IgE (PS) ( $200 \mu\text{g ml}^{-1}$ ) in 0.55 M sodium phosphate, pH 8.6, was incubated with N-glycosidase F (N-Glycanase, Genzyme Corporation),  $15 \text{ U ml}^{-1}$ , at 37 °C overnight, followed by absorption with an excess of Lentil Lectin Sepharose 4B (Sigma) at 4 °C on rotation. Analysis of the preparation by SDS-PAGE and periodic acid-Schiff staining of the gel showed that complete deglycosylation of IgE had occurred. IgE concentration in the deglycosylated sample was assessed by radioimmunoassay. The binding ability of the rE peptides was assessed by indirect immunofluorescence, as described in Table 1, incubating RPMI 8866 cells with different concentrations of rE peptides.

The binding activities of different ε-chain fragments and mutant sequences are compared in Fig. 1. The recombinant Fcε (rE2–4) is highly active, more so indeed than myeloma IgE(PS). The greater affinity is presumably due to the absence of carbohydrate, as we found that enzymatic deglycosylation of IgE(PS) led to increased activity. The peptide rE3–4, which lacks Cε2, displayed a much lower activity than rE2–4. A truncated peptide, rE2'–4, retaining only the 30 C-terminal amino acids of Cε2, was, by contrast, almost as active as the complete Fcε sequence. Thus all three Fcε domains seem to be necessary to develop full activity.

We have previously shown that Cε4 promotes the dimerization of recombinant ε-chains<sup>4</sup>. The non-covalent association between two Cε4 domains brings the sulphhydryl groups at Cys 241 and Cys 328 into register for formation of interchain disulphide bonds in Cε2. To determine whether the contribution of Cε4 to FcεR2 binding is direct or indirect, we have examined two mutant ε-chain fragments. One contains the Cε4 domain sequence, but is monomeric; the other is dimeric, but lacks the Cε4 sequence.

When Phe 506 (which lies at the interface between two Cε4 domains in Fcε, see Fig. 2) is replaced by an arginine, formation of interchain disulphide bonds is inhibited (data not shown). Monomeric and dimeric forms of rE2–4 (Arg 506) (the latter comprising <1% of the total yield) were separated and each was assayed for binding to B cells. The monomer was completely inactive, whereas the dimer was more active than the parent rE2–4 (Fig. 1). Thus FcεR2 does not recognize a monomeric ε-chain, but can bind to a site or pair of sites within a dimer.

The chimaeric immunoglobulin fragment rE2'–3/G3 contains Cys 328, which forms an interchain disulphide bond in native Fcε, and the Cγ3 domain from a mouse γ2b-chain to facilitate



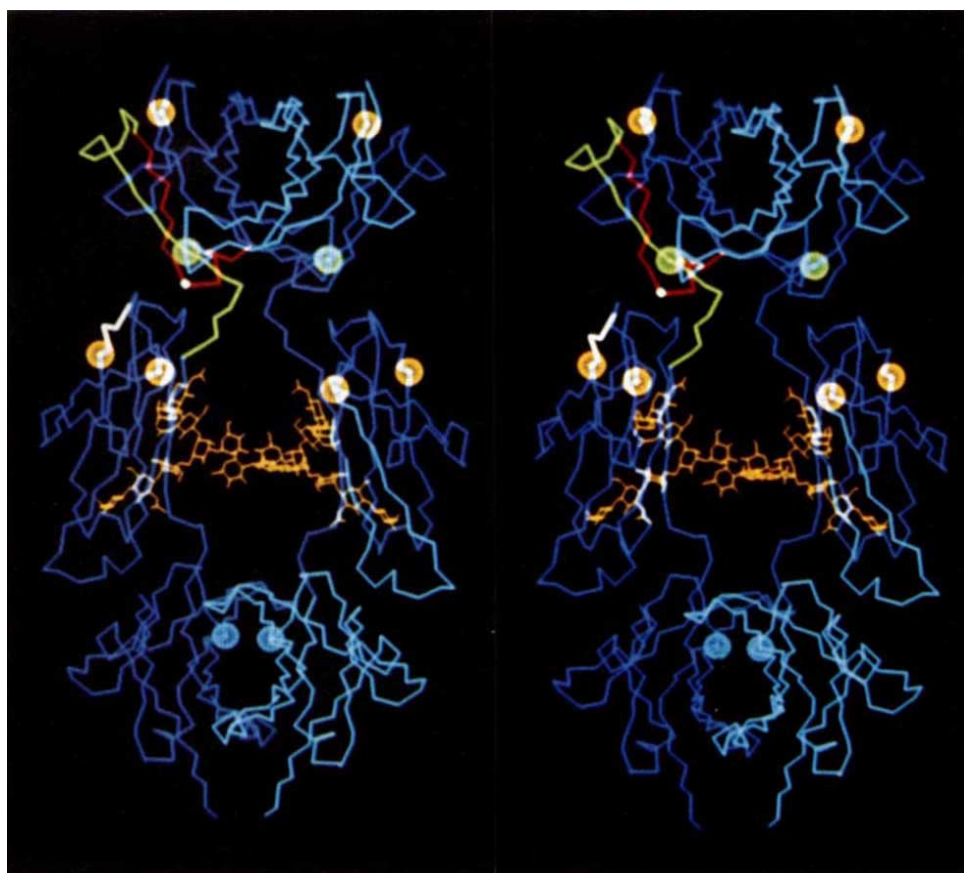


FIG. 2 Stereodrawing of the  $\alpha$ -carbon trace of a model of human IgE Fc. Model of IgE Fc (blue) showing the C $\epsilon$ 2 domains on top, the C $\epsilon$ 3 in the middle and the C $\epsilon$ 4 at the bottom. The regions corresponding to the residues Gln 301–Thr 315 are shown in red with position 307 indicated by a small white sphere. Residues Thr 315–Val 336 are shown in green, Lys 367–Val 370 in white on the left chain, and residues Arg 227–Gln 301, Leu 340–Val 361 and Arg 440–Lys 547 in light blue on the right chain. The cysteine residues at positions 328 in both chains are indicated by greenish-white dotted spheres near the bottom of the C $\epsilon$ 2 domains, the phenylalanines at 506 by light blue spheres in C $\epsilon$ 4, and the glycosylated asparagines at positions 265 near the top of C $\epsilon$ 2, and positions 371 and 394 in C $\epsilon$ 3, by yellow spheres. The carbohydrate attached to Asn 394 is shown in yellow.

dimerization (data not shown). Its activity was within an order of magnitude of that of its non-chimaeric IgE counterpart (rE2'–4), whereas mouse IgG2b had no detectable activity (data not shown). Thus C $\epsilon$ 4 is not specifically required for binding, but serves to establish a dimeric structure of the Fc $\epsilon$  that can be recognized by Fc $\epsilon$ R2.

Nine monoclonal antibodies (mAbs) against Fc $\epsilon$  were tested

TABLE 2 Specificity and inhibition of  $^{125}$ I-labelled IgE binding to Fc $\epsilon$ R2

| Anti-human Fc $\epsilon$ mAbs | Epitope location                 | % Inhibition of $^{125}$ I-IgE binding |
|-------------------------------|----------------------------------|----------------------------------------|
| DC                            | C $\epsilon$ 2 (Asn 218–Gln 301) | None                                   |
| AS 7.12                       | C $\epsilon$ 2 (Asn 218–Gln 301) | None                                   |
| BS 17                         | C $\epsilon$ 2 (Gln 307–Thr 315) | 78                                     |
| RP 3                          | C $\epsilon$ 2 (Asp 307–Thr 315) | 77                                     |
| IC 272                        | C $\epsilon$ 2 (Thr 315–Val 336) | 22                                     |
| Le 27                         | C $\epsilon$ 3 (Leu 340–Val 361) | None                                   |
| RP 1                          | C $\epsilon$ 3 (Leu 340–Val 361) | None                                   |
| IC 27                         | C $\epsilon$ 3 (Lys 367–Val 370) | 88                                     |
| IC 173                        | C $\epsilon$ 4 (Arg 440–Lys 547) | None                                   |

The specificity of the anti-Fc $\epsilon$  mAbs was deduced by their pattern of reactivity with the rE fragments<sup>4</sup>, rE fragments containing amino acids Asn 218–Thr 315 (ref. 9) and a synthetic peptide comprising amino acids Asp 307–Val 370 (provided by S. Horvath) in enzyme-linked immunosorbent assay, western blot and dot immunoassay<sup>4,10</sup>. The mAbs BS17 and Le27 were provided by B. Stadler; 7.12 and DC by A. Saxon, and D. Capra, respectively; RP1 and RP3 by R. Pumphrey; IC272, IC27 and IC173 by J. Banchereau. Purified human myeloma IgE(PS) was iodinated by the chloramine-T method (specific activity: 8,000 cpm ng<sup>-1</sup>). To test the ability of the anti-Fc $\epsilon$  mAbs to inhibit the binding of IgE to Fc $\epsilon$ R2,  $^{125}$ I-IgE (15 ng in 50  $\mu$ l) in PBS-0.5% BSA was mixed with a 10, 100, or 1,000 molar excess of anti-Fc $\epsilon$  mAbs for 1 h at 37 °C, and then added to  $1 \times 10^6$  RPMI 8866 cells in 0.1 ml. After incubation for 2 h at 4 °C, the cells were spun through serum and the cell-bound radioactivity was counted. Maximal binding was determined by incubating the cells with  $^{125}$ I-labelled IgE in the presence of medium alone. The table shows the inhibition obtained with a 10 molar excess of mAb.

for their capacity to inhibit the IgE–Fc $\epsilon$ R2 interaction, and three were found to be inhibitory. The epitopes for mAb BS17 and RP3 are contained within the C $\epsilon$ 2 sequence, Gln 307–Thr 315, and that for IC27 within the C $\epsilon$ 3 sequence, Lys 367–Val 370 (Table 2). These two sequences are in close proximity in the 3-dimensional structure of IgE (Fig. 2). The recombinant peptide binding studies suggest that the Fc $\epsilon$ R2 binding site is in C $\epsilon$ 3; BS17 and RP3 must therefore act by way of steric hindrance. The mAb inhibition results then point to a binding site in C $\epsilon$ 3 that is near to Lys 367–Val 370 in the sequence of C $\epsilon$ 3.

The Fc $\epsilon$ R2 and Fc $\epsilon$ R1 binding sites in human IgE are distinct—Fc $\epsilon$ R1 requires sequence(s) N-terminal to Leu 340 in C $\epsilon$ 3 (ref. 4), whereas Fc $\epsilon$ R2 does not. Furthermore, the mast-cell receptor binds to monomeric  $\epsilon$ -chains, whereas the B-cell receptor accepts only a dimer. This could imply that the receptor (or two receptors) must attach to both chains, or that structural constraints on the individual chains govern binding. Pre-existing receptor dimers<sup>13</sup>, with binding sites so disposed as to allow attachment to two identical sites on a single IgE molecule, would strongly favour a 2:1 complex.

It is not surprising that the sites recognized by the two IgE receptors are different as the receptors are unrelated proteins. Fc $\epsilon$ R1, in common with all other immunoglobulin receptors so far described, belongs to the immunoglobulin superfamily<sup>5</sup>. Fc $\epsilon$ R2 is unique, in that it shares homology with the carbohydrate-binding region of lectins<sup>6–8</sup>. That Fc $\epsilon$ R2 recognizes the protein moiety of IgE is therefore unexpected. □

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## Reassortment of pilin genes in *Neisseria gonorrhoeae* occurs by two distinct mechanisms

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PHASE and antigenic variation of pilin expression in *Neisseria gonorrhoeae* result from recombination events in which variant sequences from one of the silent loci (*pilS*) are transferred to the expression locus (*pilE*). Such rearrangements were originally thought to be gene conversions<sup>1–3</sup>, but findings showing that phase variation is partially inhibited by DNase I<sup>4,5</sup>, that pilated ( $P^+$ ) cells are highly competent for DNA uptake<sup>6</sup> and that gonococci readily undergo autolysis in culture<sup>7</sup>, led to the suggestion that pilin variation occurs through transformation by exogenous DNA<sup>4,8</sup>. We have developed a simple method for the selection of non-piliated ( $P^-$ ) cells and have evaluated naturally occurring  $P^+$  to  $P^-$  transitions. Two primary pathways of pilin variation can be distinguished—transformation-mediated recombination, which is influenced by culture conditions and inhibited by DNase I, and intragenomic reciprocal recombination, which is unaffected by DNase I. Furthermore, we demonstrate that both pilated and revertible  $P^-$  cells are competent for DNA uptake, an essential prerequisite of the first pathway.

Several revertible piliation phases have been described for *N. gonorrhoeae*: conventional  $P^+$  variants; S-variants, which produce a soluble secreted pilin and show intermediate degrees of piliation; and L-variants, which have a tandem duplication in the pilin structural gene and which synthesize an overlong pilin that is not assembled into pili<sup>9</sup>. Southern hybridizations and DNA-sequence analyses indicate that these variants could arise by similar recombination processes, although L-variants may also revert by deletion of one copy of the tandem duplication in the expressed gene (ref. 9, and P. A. Manning *et al.*, manuscript in preparation). In addition, deletion mutations spanning the *pilE* promoter can lead to a non-revertible  $P^-$  ( $P_n$ ) phenotype<sup>10,11</sup>, and  $P^-$  cells can also result from point mutations<sup>12</sup>. We observed that L-variants and  $P_n$  deletion mutants of *N. gonorrhoeae* strain MS11 tolerate higher levels of certain antibiotics than other variants (Table 1). The nature of this resistance phenomenon is unknown. Nevertheless, we reasoned

TABLE 1 Antibiotic resistance of *N. gonorrhoeae* MS11 pilin variants

| Piliation | Kanamycin<br>( $\mu\text{g ml}^{-1}$ ) | Penicillin<br>( $\mu\text{g ml}^{-1}$ ) | Chloramphenicol<br>( $\mu\text{g ml}^{-1}$ ) |
|-----------|----------------------------------------|-----------------------------------------|----------------------------------------------|
| $P^+$     | 5                                      | 0.3                                     | 4                                            |
| S         | 5                                      | 0.3                                     | 4                                            |
| L         | 21                                     | 2                                       | 4                                            |
| $P_n$     | 21                                     | 2                                       | 4                                            |

Cells were streaked on GC plates containing varying concentrations of antibiotics and incubated at 37 °C for 20 h. Antibiotic resistance is expressed as the minimal inhibitory concentration.

that the differential antibiotic resistance could serve as a simple means to identify and quantitate non-piliated variants. Indeed, when pilated cells were grown in culture and then plated on kanamycin ( $18.5 \mu\text{g ml}^{-1}$ ), resistant colonies were obtained. A majority (85–90%) of the colonies were found to synthesize L-pilin, whereas pilin could not be detected in lysates of the remaining colonies. The frequency of kan<sup>r</sup> colonies varied in independent experiments, depending on the culture conditions and the growth phase. In general, we observed higher switch frequencies in dense cultures than in early logarithmic cultures; autolysis and the concomitant release of chromosomal DNA may be a significant factor in dense cultures, causing this effect<sup>7</sup>.

Recent findings suggest that pilin variation is decreased, but not completely inhibited, either by DNase I (refs 4 and 5) or in a mutant defective in DNA uptake<sup>8</sup>. We therefore applied the kanamycin selection procedure to isolate non-piliated variants that had undergone pilin phase transition, either in the absence or presence of DNase I (see legend to Fig. 1). In ten independent experiments, the number of kan<sup>r</sup> variants per total CFU fluctuated between  $10^{-4}$  and  $10^{-6}$ ; this fluctuation is to be expected as phase transitions should have occurred at different times after the bacterial cells were plated. The average number of resistant colonies per CFU was fivefold lower for cells grown in the presence of DNase I, which may reflect a decreased switch frequency in the presence of DNase I ( $P \geq 0.9$  in Student's *t*-test). The values cannot, however, serve as an accurate estimate of the switch frequencies, because we are unable to evaluate the reversion rate of L-variants to  $P^+$  or S-variants, which may be high as a result of deletion of one copy of the duplication in the pilin structural gene.

L-pilin variants obtained by the kanamycin selection procedure were analysed by Southern blotting to examine the *pil* loci rearrangements (Fig. 1). We have previously demonstrated that the generation of L-variants involves the transfer of sequences from one of the silent loci (usually *pilS6*) to the expression locus, leading to a larger *pilE1* segment (ref. 9, and P. A. Manning *et al.*, manuscript in preparation). Using an oligonucleotide (RH2) that hybridizes to all known *pil* loci as a probe, L-variants obtained in the absence of DNase I were shown to possess the characteristic overlength *pilE1* locus (panel a, arrow). That this rearrangement involved transfer of sequences from the *pilS6* locus can be seen using a probe (PM007) specific for *pilS6* sequences of the parental  $P^+$  variant (panel b). The *pilS6* locus itself was not altered, nor could gross alterations in other *pil* loci be seen, suggesting that these variants were generated by an apparently non-reciprocal event, similar to the recombinations that were previously interpreted as gene

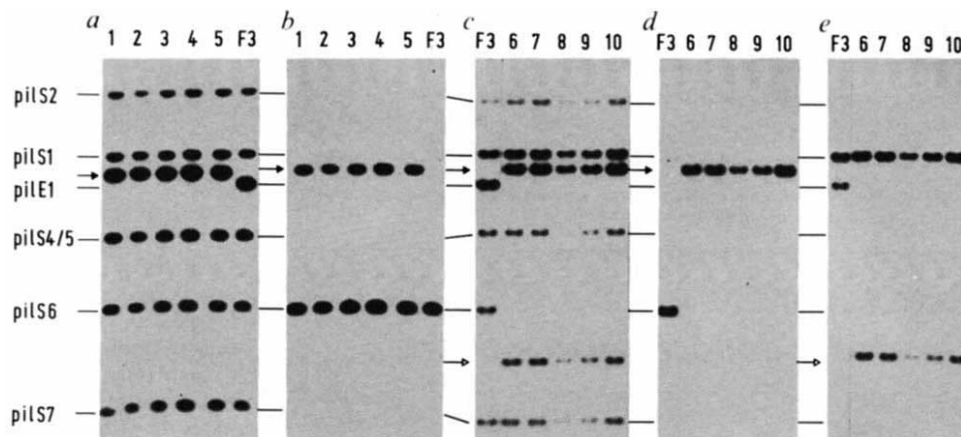
TABLE 2 Transformation competence of *N. gonorrhoeae* pilin variants

| Variant (mutant) | Piliation type | Transformation frequency | Transformation frequency (corrected) |
|------------------|----------------|--------------------------|--------------------------------------|
| F3               | $P^+$          | $1.7 \times 10^{-4}$     | $1.7 \times 10^{-4}$                 |
| B1               | S              | $5.4 \times 10^{-4}$     | $5.4 \times 10^{-4}$                 |
| B9               | S              | $2.5 \times 10^{-5}$     | ND                                   |
| D3               | S              | $2.7 \times 10^{-4}$     | ND                                   |
| D5               | S              | $8.7 \times 10^{-5}$     | ND                                   |
| D1               | L              | $1.6 \times 10^{-5}$     | $4.9 \times 10^{-6}$                 |
| (B2)             | $P_n$          | $< 5.4 \times 10^{-9}$   | $< 5.4 \times 10^{-9}$               |

Cells were suspended (absorbance at 550 nm, 0.1) in proteose-peptone medium plus 10 mM  $\text{Mg}^{2+}$  containing  $1 \mu\text{g ml}^{-1}$  linearized pC19a. Cultures were incubated at 37 °C for 4.5 h. Serial dilutions were spread on GC plates or on GC plates containing  $8 \mu\text{g ml}^{-1}$  chloramphenicol. The pilin synthesized by cam<sup>r</sup> transformants was determined by western blotting. Transformation frequency is expressed as the number of cam<sup>r</sup> colonies per CFU (average of three experiments, except for variants B9, D3 and D5); corrected values were determined by multiplying the transformation frequency by the percentage of clones synthesizing pilin identical in size to that of the parental variant. Variants of strain MS11 have been described in ref. 9, deletion mutant B2 in ref. 11. ND, not determined.



FIG. 1 Southern analyses of *kan<sup>r</sup>* pilin variants. Piliated gonococci (MS11 variant F3, ref. 9) were passaged twice on GC plates containing DNase I ( $40 \mu\text{g ml}^{-1}$ ) to minimize pilin variation. Single colonies were streaked out on normal GC plates or on GC plates containing DNase I ( $40 \mu\text{g ml}^{-1}$ ). After 24 h incubation, cells were collected with a cotton swab and suspended in 1 ml GC broth. Serial dilutions were spread on GC plates containing kanamycin ( $18.5 \mu\text{g ml}^{-1}$ ) and DNase I ( $40 \mu\text{g ml}^{-1}$ ) for selection, or on normal GC plates to determine the total CFU. Genomic DNA from L-variants selected on kanamycin was digested with *Cla*I and hybridized to end-labelled oligonucleotides specific for different *pil* loci. DNAs: F3, parental variant; lanes 1–5, L-variants obtained in the absence of DNase I; lanes 6–10, L-variants obtained in the presence of  $40 \mu\text{g ml}^{-1}$  DNase I. Probes: panels a, c, RH2 (CGGCTTGCTTTAAGGGTTTGAAGG); panel



b, PM007 (GGGTAATACCCGGTGATGGCTGATTT); panel d, RH12 (TTTCTTTGTC-TTTCGCCGGTGGTGTCCG); panel e, RH6 (TTTTCGTTGTCGTTGCCGGCTTTGGT).

conversions<sup>1-3</sup>. L-variants obtained in the presence of DNase I showed a different hybridization pattern with probe RH2 (panel c). In addition to the 500-base pair (bp) longer *pilE1* locus (closed arrow), these cells show a 500-bp deletion in *pilS6* (open arrow). This pattern suggested that reciprocal recombination took place between *pilS6* and *pilE1*. This was confirmed by hybridizations with oligonucleotide probes specific for the two loci in the parental *P<sup>+</sup>* variant. The probe RH12 specific for the parental *pilS6* locus (panel d) hybridized to the extended *pilE1* locus in the daughter L-variants, but not to the altered *pilS6* locus. Instead, the probe RH6 specific for the expressed pilin gene of the parental variant (panel e) hybridized to the altered *pilS6* locus of these variants, but not to the extended *pilE1* locus.

From these data we conclude that two distinct pathways can lead to recombination between *pilS* and *pilE* loci, resulting in pilin variation *in vitro*. One pathway has the appearance of a gene conversion event, but it can be inhibited by DNase I, and is probably the result of a transformation-dependent recombination. The second pathway, which results in a different hybridization pattern, can be interpreted as reciprocal recombination between a silent and the expression locus of the same chromosome, and is detected when the first pathway is blocked by DNase I.

Transformation-mediated recombination requires that revertible pilin variants are competent for DNA uptake. Here we have examined *P<sup>+</sup>* to *P<sup>-</sup>* transitions, and it is well established that piliated gonococci are transformable<sup>6</sup>. If transformation is involved in the reverse (*P<sup>-</sup>* to *P<sup>+</sup>*) transitions, revertible non-piliated cells, that is, L- and non-piliated S-variants, must also be transformation competent. It has been reported, however, that non-piliated cells transform with extremely low efficiency<sup>6</sup>. We re-evaluated this problem by measuring the transformation frequencies of different gonococcal pilin variants and *P<sub>n</sub>* deletion mutants (Table 2), using a cloned MS11 *recA* gene in which part of the coding region has been replaced with a selectable CAT marker (pC19a). Gonococci transformed by this construct become simultaneously *cam<sup>r</sup>* and *recA<sup>-</sup>* on recombination with the chromosomal *recA*. Because pilin variation (with the exception of point mutations) is *recA* dependent<sup>12</sup>, further pilin variation is inhibited and the piliation phase of a given cell remains unaltered. As a result, true transformants of a given variant can be distinguished from switch variants that were generated before the transformation and which may have been more efficiently transformed than the starting variant. Confirming earlier reports<sup>6</sup>, conventional *P<sup>+</sup>* variants were transformable at high frequencies and S-variants, even those that have extremely few pili<sup>9</sup>, were transformed at slightly higher frequencies. Non-piliated deletion mutants (*P<sub>n</sub>*) could not be transformed to chloramphenicol resistance but the non-piliated revertible L-

variants<sup>9</sup> were transformable, although at a lower frequency than piliated cells. Only 30% of these transformants synthesized L-pilin; the remainder were *P<sup>+</sup>* or S-variants, and probably do not represent true L-variant transformants. Nevertheless, these data indicate that transformation-mediated recombination could have a role not only for variation of piliated variants, but also for revertible non-piliated variants.

Our findings support the model of transformation-mediated pilin variation as the main mechanism of variation observed *in vitro*<sup>4,8</sup>, but we demonstrate that intragenomic recombinations also occur. Whether, or under which conditions, the two pathways are used *in vivo* during the course of a natural infection is unknown. Within the host, gonococci encounter a variety of different microenvironments; under conditions of high cell density, transformation-mediated recombination may be favoured, but at other times, reciprocal recombination could be the dominant mechanism of pilin variation. □

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## Fibronectin binding mediated by a novel class of surface organelles on *Escherichia coli*

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GRAM-negative bacteria are known to produce two types of surface organelles: flagella, which are required for motility and chemotaxis, and pili (fimbriae), which play a part in the interaction of bacteria with other bacteria and with eukaryotic host cells. Here we report a third class of *E. coli* surface organelles for

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which we propose the name curli. Curli are coiled surface structures composed of a single type of subunit, the curlin, which differs from all known pilin proteins and is synthesized in the absence of a cleavable signal peptide. Although the gene encoding this structural subunit, *crl*, is present and transcribed in most natural isolates of *E. coli*, only certain strains are able to assemble the subunit protein into curli. This assembly process occurs preferentially at growth temperatures below 37 °C. The ability of curli to mediate binding to fibronectin may be a virulence-associated property for wound colonization and for the colonization of fibronectin-coated surfaces.

Our investigation of the virulence-associated properties of *E. coli* isolates from cases of bovine mastitis revealed that approximately half the bovine mastitis (34 out of 62) and fecal isolates (22 out of 40) were able to bind soluble [<sup>125</sup>I]-labelled fibronectin. This binding was preferentially expressed when the cells were grown on colonization factor antigen (CFA)-agar at temperatures between 26 °C and 32 °C. Electron microscope analysis of 40 fibronectin-binding *E. coli* isolates revealed that they all produced coiled surface structures when grown on CFA-agar at 26 °C (Fig. 1a). High magnification of these structures, subsequently termed curli, showed them to be thin, wiry fibres with a diameter of ~2 nm (Fig. 1b). The lateral aggregation of individual fibres produced considerably thicker structures.

When 15 non-binding isolates were examined, only one, was found to produce surface structures of similar morphology.

To determine whether fibronectin binding was associated with the production of curli, a genomic cosmid gene bank from a fibronectin-binding bovine *E. coli* isolate, AO12, was screened in *E. coli* HB101. Of 560 cosmid clones tested, only one was detected which bound soluble fibronectin. This clone expressed coiled surface organelles indistinguishable from the curli found on AO12. A series of genetic constructs were then created to define the region required for fibronectin binding and for the formation of curli. A 1.5-kilobase (kb) *Sph*I-*Bgl*II fragment present on pCRL20 (Figs 1c, 2) mediated both phenotypes and encoded a unique polypeptide of relative molecular mass ( $M_r$ ) 17,000 (17K) that could be detected in minicells (Fig. 3a). The *Sph*I-*Hpa*I subclone pCRL56 (Fig. 2) produced a polypeptide slightly larger than 17K (Fig. 3a) suggesting that the corresponding gene must be transcribed from left to right and have its 3' end distal to the *Hpa*I site. An in-frame fusion between the coding sequence for the 17K protein and vector sequences would account for the increased size of the protein detected (data not shown). Insertion of an aminoglycoside 3'-phosphotransferase (APH) gene into the *Cla*I<sub>1</sub> site to give pCRL59 (Fig. 2), abolished the expression of both curli and fibronectin binding and eliminated the 17K polypeptide (Fig. 3a). Furthermore, two

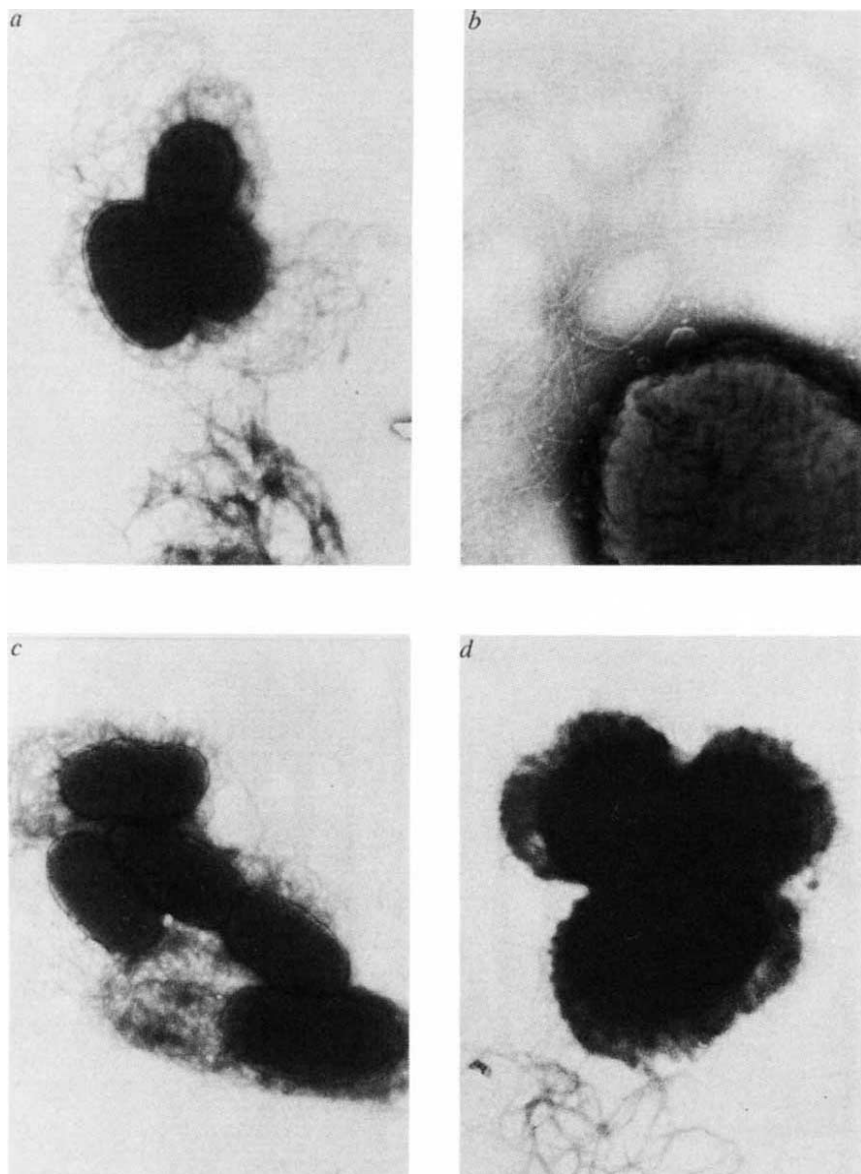


FIG. 1 Electron micrographs of negatively stained *E. coli* strains carrying the indicated recombinant plasmids: a, AO12 (natural bovine isolate); b,  $\times 60,800$  magnification of AO12 showing curli as thin, wiry organelles and the lateral aggregation of individual fibres into thicker structures; c, HB101/pCRL20; d, AO12/pCRL20. Magnification for a, c and d is  $\times 15,200$ .

**METHODS.** Electron microscopy was performed with a JOEL 100B microscope with 100-mesh copper grids coated with thin films of 2% Formvar. Bacteria were grown on CFA-agar plates at 26 °C. Cells were then resuspended in 10 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub> and placed on the grid. Grids were washed with buffer and negatively stained for 5 s with 3.55% ammonium molybdate and then washed with re-distilled water.

deletion derivatives, pCRL30 and pCRL46, were similarly unable to bind fibronectin, produce curli or express the 17K protein (Figs 2 and 3a). Taken together, these results indicate that one gene, which we have denoted *crl*, is responsible for fibronectin binding and curli production.

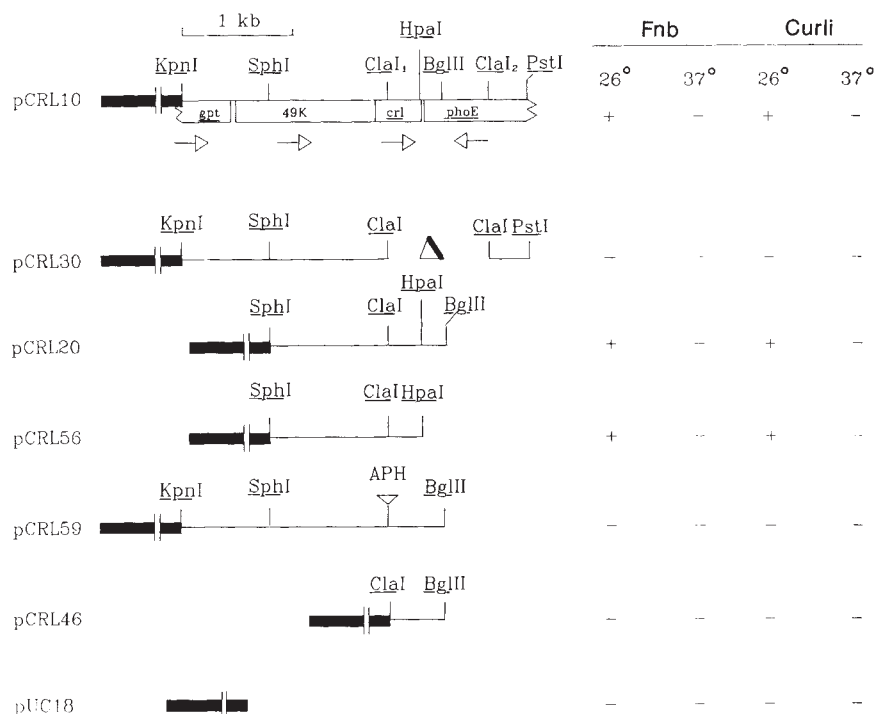
DNA sequence analysis of M13mp18 and M13mp19 deriva-

tives of pCRL20 showed that *crl* spans the *Clal*<sub>1</sub> and *HpaI* sites of this plasmid and encodes a polypeptide of 133 residues having a calculated molecular weight of 15.7K (data not shown).

The *crl* gene of the bovine isolate AO12 was found to be almost identical to an open reading frame previously detected adjacent to the *phoE* gene of *E. coli* K12 (ref. 1). A single

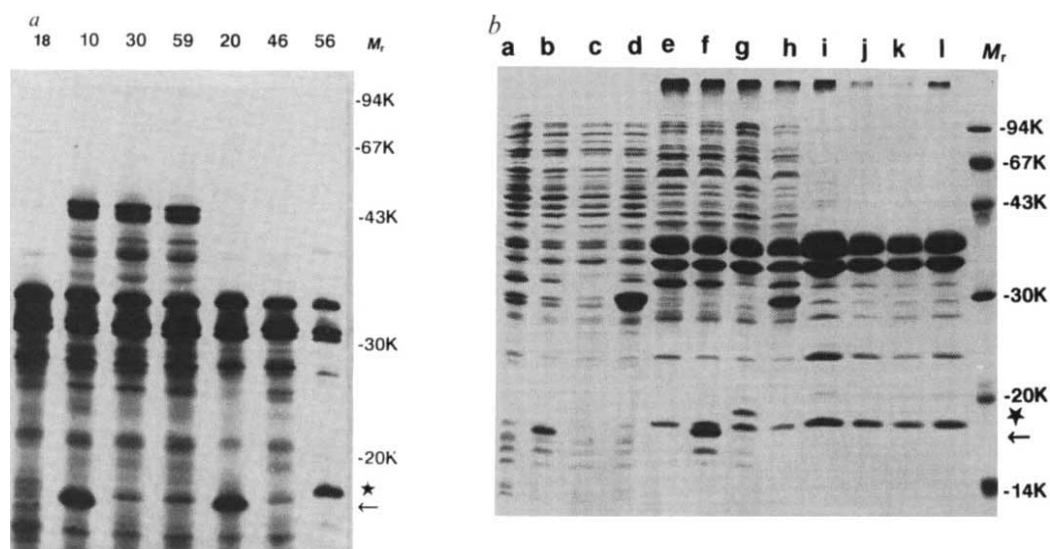
FIG. 2 Restriction map and genetic organization of the *crl* region of *E. coli*. Arrows indicate the direction of transcription of the various genes. The phenotypes Fnb and Curli represent binding to soluble [<sup>125</sup>I]-fibronectin and production of curli as measured by electron microscopy, respectively. Other abbreviations (top of figure): *gpt*, structural gene for guanine-hypoxanthine phosphoribosyl transferase; 49K, open reading frame encoding a polypeptide of unknown function; *crl*, the structural gene for curlin; and *phoE*, the structural gene for phosphate-limitation, inducible, outer-membrane pore protein. Scale bar, 1 kb.

METHODS. A cosmid library from the bovine isolate AO12 was generated in *E. coli* HB101. One cosmid clone (pAO450) mediated binding to soluble fibronectin. A 4.9 kb *SalI* fragment subcloned in pCRL1 encoded the binding ability. This *SalI* fragment was subsequently found to be equivalent to the 4.9 kb *SalI* fragment cloned from the Clarke and Carbon collection onto pJP14<sup>1,7,10</sup>. Subclones from pCRL1 were then generated: pCRL10 consists of the 3.0 kb *KpnI*-*PstI* fragment from pCRL1 ligated into the polylinker cloning cassette of pUC18; pCRL30 is a *Clal*<sub>1</sub>-*Clal*<sub>2</sub> deletion derivative of pCRL10; and plasmid pCRL20 was obtained by cloning the 1.5 kb *SphI*-*BglII* fragment from pCRL1 into pUC18. Analysis of this construct, which lacks the 5' end of the gene encoding the 49K polypeptide, showed that this protein was not necessary for curli production or for fibronectin binding. Plasmids pCRL56 and pCRL46 are *SphI*-*HpaI* and *Clal*-*BglII* subclones, respectively, of pCRL10 in pUC18. Plasmid pCRL59 was obtained by subcloning the *KpnI*-*BglII* fragment of pCRL1 into pUC18 and then inserting the aminoglycoside 3'-phosphotransferase gene (*APH*) from the mobilization plasmid pUC-4K into the *Clal*<sub>1</sub> site. To perform fibronectin-binding assays<sup>11</sup>, bacteria were grown on CFA-plates for 42-48 h at 26°C or 37°C. Cells were resuspended in ice-cold PBS plus 0.1% Tween 80 to a density of 10<sup>8</sup> c.f.u. ml<sup>-1</sup> and [<sup>125</sup>I]-fibronectin (specific activity 0.19 MBq µg<sup>-1</sup>) added



to the mixture ( $5 \times 10^5$  c.p.m. ml<sup>-1</sup>) which was then incubated end-over-end at room temperature for 1 h. After centrifugation, the supernatant fluid was carefully aspirated and the radioactivity in the pellet measured in a scintillation counter. Isolates were regarded as positive binders if they bound more than 5,000 c.p.m. of the total radioactivity added ( $\geq 10\%$ ), whereas non-binding isolates showed less than 500 c.p.m. ( $\leq 1\%$ ) binding. Background was also less than 500 c.p.m. Bovine fibronectin (Sigma) was iodinated by the peroxidase method<sup>12</sup>.

FIG. 3 a, Proteins expressed by different recombinant plasmids in the *E. coli* minicell-producing strain AA10 (ref. 9). The plasmids used, from left to right, were pUC18, pCRL10, pCRL30, pCRL59, pCRL20, pCRL46 and pCRL56. The arrow indicates the 17K *crl* product, whereas the star indicates the slightly larger fusion polypeptide expressed by pCRL56. b, Fractionation of *E. coli* AA10 cells expressing the cloned curlin: lanes a-d, whole cells; lanes e-h, total membrane fraction; and lanes i-l, outer membrane fraction. No *crl* product was found in either the cytoplasmic or periplasmic fractions (data not shown). Lanes a, e and i, AA10/pUC18; lanes b, f and j, AA10/pCRL20; lanes c, g and k, AA10/pCRL56; lanes d, h and l, AA10/pCRL59. *M<sub>r</sub>*, molecular mass standards.



fractionation was performed according to Achtman *et al.*<sup>14</sup>. To obtain an outer membrane preparation, the total membrane fraction was treated with Sarkosyl (1.5%). Samples, including low molecular mass standards (Pharmacia), were separated by 15% SDS-PAGE. Gels were fixed, stained, dried and exposed to X-ray film (Du Pont) for 1-5 days.



nucleotide difference at codon 29 of *crl* resulted in the substitution of Lys for Glu. We have established that the *E. coli* K12 *crl* gene cloned on plasmid pJP14 (ref. 1) mediates both fibronectin binding and curli production when present in *E. coli* HB101 (data not shown). Hence, the single amino-acid substitution does not affect either phenotype. We conclude that *crl* is located at 5.8 min on the *E. coli* chromosome between *phoE*, the gene for a phosphate-limitation, inducible, outer-membrane protein, and a gene encoding a 49K protein of unknown function that is adjacent to the *gpt* structural gene for guanine-hypoxanthine phosphoribosyl transferase<sup>2</sup>.

Significantly, expression of curli in *E. coli* HB101 cannot be due to complementation from chromosomal genes near *crl* as the *phoE-gpt* region is deleted in this strain as a result of its *proA2* allele<sup>3</sup>. Because this deletion removes the coding sequence for the 49K protein, we believe this protein to be inessential to the biogenesis of curli. It is possible, however, that there exist non-linked genes, as yet unidentified, that do encode biogenesis functions.

A partially purified preparation of curli from HB101/pCRL20 was found to consist of a dominant protein of apparent  $M_r$  17K (by SDS-PAGE, data not shown). The 17K protein was electroeluted onto an Immobulon filter<sup>4</sup> and the N-terminal sequence determined by Edman degradation<sup>5</sup>. The five detected N-terminal residues (Thr-Leu-Pro-Ser-Gly) of the 17K protein were identical to those deduced from codons 2–6 of the sequenced DNA<sup>1,2</sup>, confirming that *crl* encodes the subunit protein for curli.

The curlin subunit differs from *E. coli* pilins in several respects. Features common to the pilins include a cleavable signal peptide, two Cys residues in their N-terminal region and many conserved amino acids, particularly in their N- and C-terminal sequences which are thought to be involved in subunit-subunit interactions<sup>6</sup>. Curlin shares none of these features. Furthermore, no homologies were found between curlin and the *N*-methylphenylalanine class of pili expressed by *Neisseria gonorrhoeae* and many other Gram-negative species<sup>7</sup>.

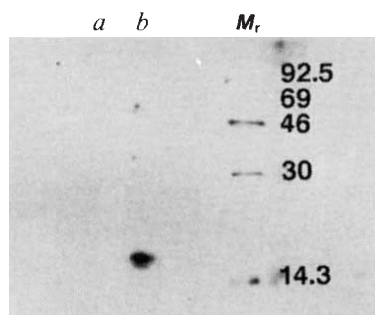


FIG. 4 Binding of curlin to [<sup>125</sup>I]-fibronectin. A partially purified preparation of curli was obtained from HB101/pCRL20. As a control, HB101/pCRL30 (*crl*<sup>-</sup>) was subjected to the same treatment. Lane a, pCRL30; lane b, pCRL20;  $M_r$ , molecular mass standards (K). [<sup>14</sup>C]-methylated proteins (Amersham): 92.5, phosphorylase b; 69.0, bovine serum albumin; 46.0, ovalbumin; 30.0, carbonic anhydrase; 14.3, lysozyme.

**METHODS.** To purify curli, strain HB101/pCRL20 was grown on CFA-agar containing carbenicillin for ~48 h at 26 °C. Cells were collected into ice-cold Tris buffer and blended in a Sorvall Omnimixer for 4 × 2 min at maximum speed. Bacterial cells were removed by centrifugation at 15,000 *g* for 15 min and curli then precipitated in 0.1 M MgCl<sub>2</sub>, 0.15 M NaCl, 0.05 M Tris, pH 7.0 and collected by centrifugation at 13,000 *g* for 15 min. Successive cycles of solubilization and precipitation were used to obtain a partially pure curli preparation. Samples were separated on a 15% SDS-PAGE and transferred to Immobulon filter using a Trans-Blot SD electroblotter (BIO-RAD) and the filter was blocked with TSB buffer (140 mM NaCl, 10 mM Tris, pH 7.5, 0.1% Tween 80) for 2 h at room temperature. A protein-blot ligand assay was performed with [<sup>125</sup>I]-fibronectin (specific activity 0.19 MBq μg<sup>-1</sup>) in TSB buffer for 12 h. The filter was rinsed repeatedly in excess TSB buffer, dried, and autoradiographed for 6 days at -80 °C using Amersham Hyperfilm-MP with an intensifying screen.

To demonstrate directly the binding of fibronectin to the curlin subunit, curli prepared from HB101/pCRL20 were electrophoresed under reducing conditions on SDS-PAGE, electroeluted onto Immobulon filter and incubated with [<sup>125</sup>I]-fibronectin. Specific binding of the fibronectin to a 17K polypeptide indicative of the curlin protein was detected (Fig. 4, lane b). No binding was seen to proteins released from HB101/pCRL30, which lacks the *crl* gene (Fig. 4, lane a). It is not yet known, however, which region of the curlin subunit binds to fibronectin, nor which domain in the fibronectin molecule interacts with curli.

The presence of *crl* in *E. coli* K12 prompted us to analyse several K12 strains for the production of curli and for their ability to bind fibronectin. Strain C600<sup>8</sup> was found to bind fibronectin and to produce curli when grown on CFA-agar at 26 °C. By contrast, the *E. coli* K12 minicell-producing strain AA10<sup>9</sup>, which carries the *crl* region as determined by DNA-DNA hybridization using the 0.3 *Cl*<sub>1</sub>-*Hpa*<sub>1</sub> fragment (Fig. 2) as a *crl*-specific probe (data not shown), did not express curli or bind soluble fibronectin. Furthermore, when various *crl* clones were transformed into this strain (for example, AA10/pCRL10 or AA10/pCRL20), the expression of the cloned 17K *crl* gene product was detected at both 26 and 37 °C in minicells and in whole-cell extracts (Fig. 3a, b). These findings demonstrate that although *E. coli* K12 AA10/pCRL20 can express the *crl* gene product, it cannot assemble the curlin into curli. To localize the unassembled curlin, AA10/pCRL20 and AA10/pCRL56 were fractionated into their subcellular compartments (Fig. 3b). Plasmid pCRL56 expresses the slightly larger form of curlin which results from an in-frame fusion with vector DNA, and this enabled us to distinguish between a plasmid-encoded *crl* product and a possible chromosomally encoded *crl* gene product of AA10. Curlin was located in the total membrane fraction of these strains (Fig. 3b, lanes f, g) but not in the outer membrane fraction (Fig. 3b, lanes j, k). As curlin seemed to be entirely solubilized by the Sarkosyl extraction, we conclude that the unassembled protein must be located in the cytoplasmic membrane.

A *crl*-specific probe hybridized with either a 5.0 kb or 3.0 kb *Sal*<sub>I</sub> chromosomal fragment in all bovine *E. coli* strains tested, irrespective of their ability to bind fibronectin. Furthermore, northern blot analysis showed that the *crl* gene is equally transcribed in strains that are proficient for curli production and fibronectin binding as in those that are not (data not shown). When the *crl* plasmid pCRL20 was introduced into non-binding mastitis isolates, the transformants did not acquire the ability to produce fibronectin-binding curli. By contrast, when pCRL20 was introduced into the original isolate AO12, there was a dramatic increase in the production of curli that covered the cell surface as a dense coat (Fig. 1d). These results suggest that although most *E. coli* strains carry a transcribable *crl* gene, only a subset of isolates can assemble the curlin subunit into the novel surface structures identified as curli. □

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# Interaction of distinct domains in Mu transposase with Mu DNA ends and an internal transpositional enhancer

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**BACTERIOPHAGE Mu is the largest and most efficient transposable element known<sup>1</sup>. The Mu transposase (A protein) of relative molecular mass 75,000 is a central component of the transposition machinery<sup>2</sup>. We report here that the N-terminal region of Mu transposase contains two distinct DNA-binding domains, one which binds the two Mu DNA ends, and another which binds an internal operator region. This internal operator is required for the transposase-mediated synapsis and nicking of Mu ends *in vitro*, and stimulates transposition more than 100-fold *in vivo*. The orientation of the operator with respect to the ends is critical to its function, whereas its distance from the ends seems to be relatively**

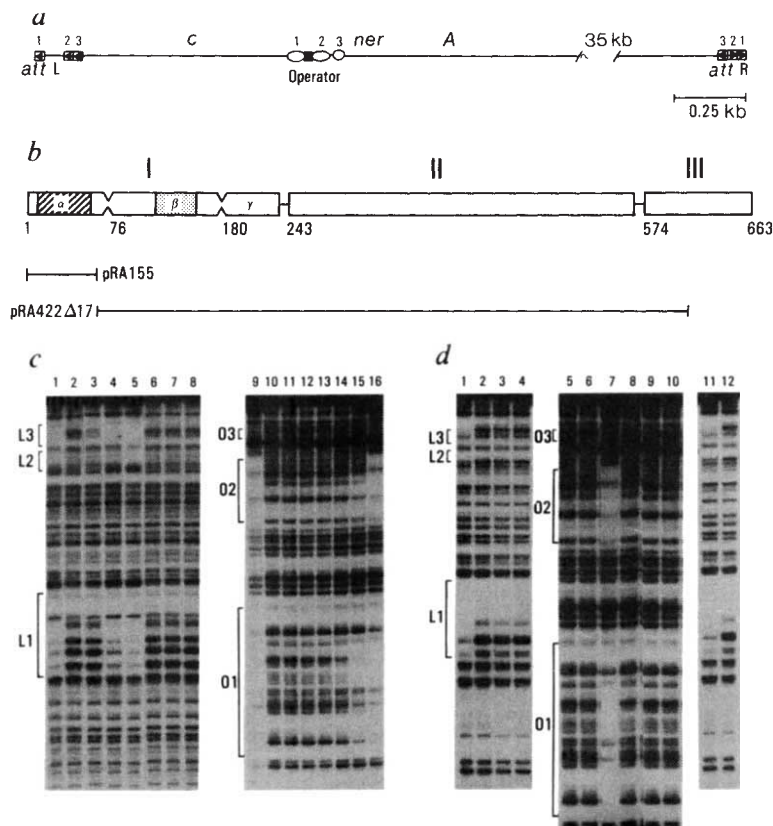
unimportant. We propose that the operator enhances transposition by transiently interacting with the transposase and Mu DNA end(s) to form a complex in which synapsis of the ends occurs.

The structural organization of the Mu genome and the transposase polypeptide<sup>3</sup> is presented in Fig. 1a and b. The DNA-binding activities of domains of the transposase were studied using the truncated polypeptide AΔ17 which is a transposase mutant missing 62 residues at the N-terminus (most of domain Iα; Fig. 1b) and 55 residues at the C-terminus. Purified AΔ17 bound to *attL* and *attR*, but not to the operator (Fig. 1c, lanes 3–5 and 11–13; data for *attR* not shown). A subsequent deletion analysis mapped the *att* DNA-binding region of A to domain Iβ, between residues 77 and 177 (data not shown), and the operator-binding region of A to domain Iα, in its first 64 amino acids (Fig. 1c, lanes 6–8 and 14–16). Thus, subdomains Iα and Iβ of the transposase bind to two different classes of DNA sequence.

Can domains Iα and Iβ simultaneously bind the two sets of DNA sites? The preincubation of A with an excess of *attL* DNA fragment abolished binding to an operator DNA fragment (Fig. 1d, lanes 5 and 7), implying that Iα cannot bind the operator stably when Iβ is occupied with *attL*. In a reverse experiment, however, the preincubation of A with excess operator did not prevent binding to *attL* (Fig. 1d, lanes 1 and 11); similar results were obtained with *attR* (data not shown). To determine whether the latter results were due to a combination of an instability of the operator bound to Iα and a higher affinity of Iβ for *att* DNA (estimated to be two- to fourfold; data not shown) a gel-retardation assay was performed to examine the stability of

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FIG. 1 a, Mu DNA regions important in transposition. The Mu DNA ends are oriented inversely on a negatively supercoiled DNA molecule during transposition<sup>19</sup>. Boxes at *attL* and *attR* are A-binding sites. Arrowheads inside the boxes indicate orientation of a consensus recognition sequence<sup>9</sup>. The order of A-binding affinities is: L1, L3, R3 > R2 > L2, R1. The Mu repressor *c* also binds to these sites, but with lower affinity. Ellipses represent operator sites bound by repressor and also by A (refs 9, 20). The order of affinity of both proteins for operator sites is O1 > O2 > O3 (see ref. 20 and c). A binding site for *E. coli* IHF exists between O1 and O2 (filled-in square)<sup>20</sup>. *Ner* binds between O2 and O3 (ref. 21). Not shown are two divergent promoters in the operator area for leftward (gene *c*) or rightward (early) gene transcription. The lysis-lysogeny decision is apparently controlled by the impact on transcription of the regulatory proteins competing for the various DNA-binding sites (see ref. 7). b, Structure of A. The A protein contains three domains; site-specific *att* DNA-binding activity resides in the N-terminal 30 k domain I (ref. 3). This domain contains three subdomains (α, β and γ). Shaded areas in Iα and Iβ share regions of homology with the repressor<sup>2</sup>. Amino-acid residue numbers are shown beneath the structure. The length of A protein encoded by plasmids pRA155 (A-*lacZ* fusion containing A DNA between *EcoRV* and *DdeI* sites (1,307–1,514; ref. 2) cloned in-frame with the entire *lacZ* gene into pUC8; 64 N-terminal A residues fused to β-galactosidase) and pRA422Δ17 (AΔ17; ref. 22), containing Mu DNA starting from the *HindIII* site in the immunity region and carrying a deletion of 165 bp from the 3' end of A, and a deletion of T at position 1,512 (ref. 2) at the 5' end of A, cloned under λ P<sub>L</sub> promoter on pKC30; protein missing 62 residues at the N-terminus and 55 at the C-terminus) is indicated below the domain structure. In pRA422Δ17, the deletion of the T at position 1,512 created an internal ATG codon at which AΔ17 protein initiated. The N-terminal sequence of purified AΔ17 was determined to be MLRQGEIETSLGYF. When reconstituted with the wild-type sequence at its N-terminus, AΔ17 was active in transposition both *in vivo* and *in vitro* showing that the C-terminal deletion does not interfere with the DNA-strand transfer activity of A (ref. 22 and unpublished observations). This deletion however, leaves the protein 'blind' to Mu B. c, Nuclease footprints of A protein fragments on *attL* and operator DNA. Lanes 1–8 contain ~0.1–1 nM 5'-labelled *attL* fragment from pRA-L29 (ref. 3) digested with DNAaseI. Before digestion, the *attL* fragment was incubated with 0.67 μM A (lane 1), no protein (lane 2), 0.4, 0.8, and 1.2 μM AΔ17 (purified as described in ref. 23) from pRA422Δ17 (lanes 3–5) or 0.3, 0.6 and 1.2 μM A-β-galactosidase fusion protein (purified up to the ammonium sulphate precipitation step as previously described<sup>23</sup>; the precipitate was dissolved in 50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, and affinity-purified using p-aminobenzyl-1-thio-β-D-galactopyranoside-agarose<sup>24</sup> from Sigma) from pRA155 (lanes 6–8). Lanes 9–16 contain ~0.1–1 nM 5'-labelled operator fragment from pHK09 (ref. 20) digested with Neocarcinostatin (NCS), treated as in lanes 1–8. Reaction products were electrophoresed on 6% polyacrylamide gels, as previously described<sup>9</sup>. Regions



protected by A are indicated in the left margins and correspond to sites shown in a. d, Competition nuclease protection experiments for *attL* and operator DNA. All lanes were treated with NCS. Lanes 1 and 3 show footprints of A (67 nM) on *attL* (0.1 nM) in the absence or presence, respectively, of a 100-fold molar excess of unlabelled *attL* fragment. Lanes 2 and 4 are identical to 1 and 3 but contain no A protein. Lanes 5 and 6 are identical to 3 and 4 except that the labelled fragment is operator. Lanes 7 and 9 show footprints of A (67 nM) on operator (0.1 nM) in the absence or presence, respectively, of a 100-fold molar excess of unlabelled operator. Lanes 8 and 10 are identical to 7 and 9 but contain no A protein. Lanes 11 and 12 are identical to 9 and 10 except that the labelled fragment is *attL*. Other descriptions are as in c.

the A-operator complex (Fig. 2a). When a preformed complex (lane 2) was challenged with excess operator DNA, a significant portion of the DNA in the complex was released quickly (lane 3), whereas the rest remained stably complexed with the protein for up to 40 minutes (lanes 3–5). Challenging with *attL* DNA, however, caused rapid and complete dissociation of the A-operator complex (lanes 6–8). Qualitatively similar results were obtained with *attR* (lanes 9–11), although it was less efficient at dissociating the A-operator complex. (In a control experiment, an operator complex between the pRA155-encoded  $I\alpha$  domain alone failed to bind *att* DNA or to dissociate in the presence of *att* DNA; data not shown.) By contrast, the preformed complex between A and *attL* was significantly more resistant to challenge by either *attL* or operator DNA (Fig. 2b, lanes 1–8). Here the competitor DNAs modified the number of A moieties in the complex, but caused little or no release of the A-bound *attL* site over a period of 40 minutes. These results

show that prior binding of the  $I\beta$  domain of A to the *att* sites prevents stable binding of the  $I\alpha$  domain to the operator. Conversely, preformation of an  $I\alpha$ -operator complex does not prevent  $I\beta$  from efficiently binding *att*, after which the operator is released. *AttL* is more efficient than *attR* at inducing the release of operator from the complex. Thus, a ternary complex of A, an *att* site, and an operator, if it exists, must be short-lived.

To study the role of the operator-binding region in transposition, we varied the location, orientation, and number of the  $I\alpha$ -binding sites in a set of seven mini-Mu plasmids. We then tested the ability of these plasmids to react with A protein, *Escherichia coli* protein HU, and  $Mg^{2+}$  to form type I transpososome complexes, in which the Mu DNA ends synapse and the 3' ends of *attL* and *attR* are nicked<sup>4,5</sup>. Moving the operator, which is normally 1 kilobase (kb) from *attL*, to 0.45 kb or 2.2 kb from *attL* had no effect on the ability of Mu ends to synapse and form the type I complex (Fig. 3; pRA167, pRA170, and

FIG. 2 Dissociation of A-*attL* and A-operator complexes monitored by gel-retardation. **a**, About 0.05 nM 5'-labelled operator DNA (lane 1) was complexed with 67 nM A for 20 min (lane 2) and then challenged for 0.5, 10 and 40 min, respectively, with a 100-fold molar excess of either unlabelled operator, (lanes 3–5), unlabelled *attL* (lanes 6–8) or unlabelled *attR* (lanes 9–11). Reactions were performed and products electrophoresed on 6% polyacrylamide gels as previously described<sup>9</sup>. The *attR* fragment is from pPLR1, derived by cloning a filled-in *RsaI* fragment from pCN201 (ref. 25), which includes 200 bp of Mu right-end, into the *SmaI* site of pUC8. I, II and III are complexes that presumably contain one, two or three of the operator sites occupied by A. **b**, About 0.05 nM 5'-labelled *attL* DNA (lane 1) was complexed with A as above (lane 2) and then challenged for 0.5, 10 and 40 min, respectively, with a 100-fold molar excess of either unlabelled *attL* (lanes 3–5), or unlabelled operator (lanes 6–8). All other descriptions are as in **a**.

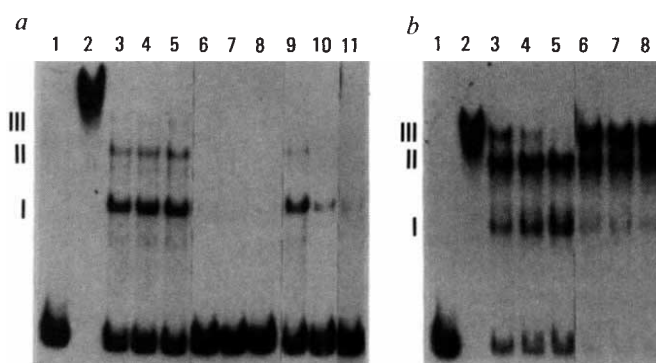
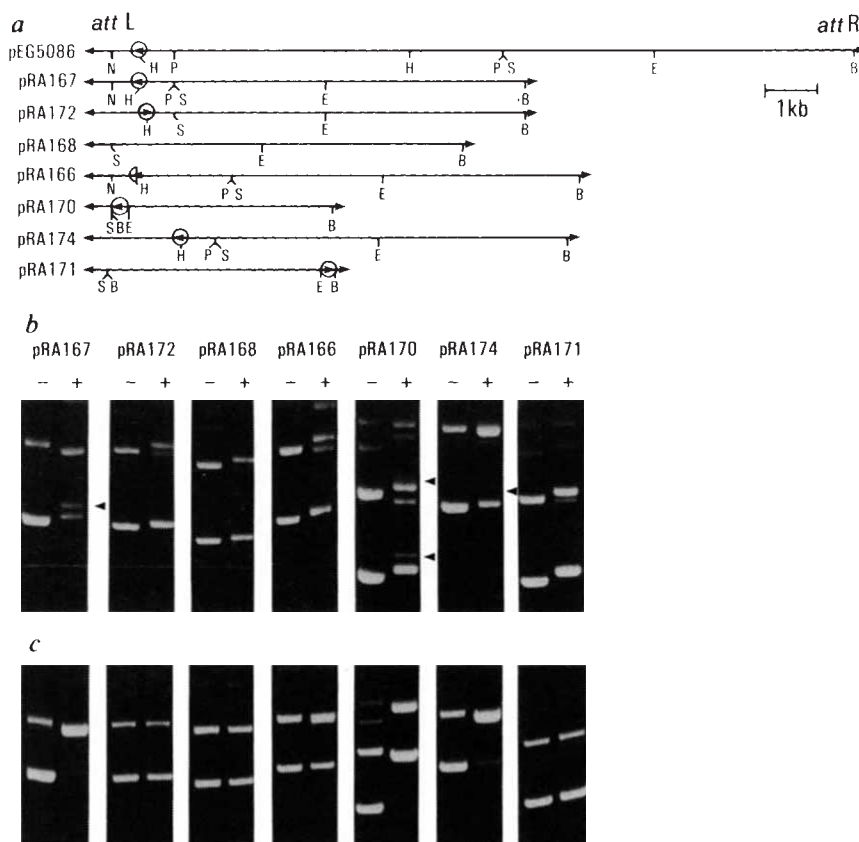


FIG. 3 **a**, Map of mini-Mu sequences in various plasmids. All plasmids were derived from pEG5086 (ref. 26). The length of the DNA outside the Mu ends is ~2.2 kb (not shown). pRA166 is a *HindIII* (H) dropout, pRA167 a *PstI* (P) dropout and pRA168 a *NsiI*(N)-*PstI* dropout of pEG5086. pRA170 has *SalI*(S)-*EcoRI*(E) operator fragment from pHK09 (ref. 20), cloned into pRA168. In pRA171 the *BamHI*(B) fragment of pRA170 is inverted. In pRA172 the *NsiI*-*PstI* fragment of pRA167 is inverted. pRA174 has a 1.2 kb *Kan<sup>R</sup>* cassette (*PstI* fragment from pUC-4K, Pharmacia) inserted into the *NsiI* site of pRA167. The circled arrow signifies the operator (which includes all three sites), and has been assigned an arbitrary direction. In pRA166, 01 and part of 02 are intact<sup>20</sup>. **b**, Formation of type I transpososome complexes. Mini-Mu plasmid DNA ( $50 \mu\text{g ml}^{-1}$ ) was treated with *E. coli* HU protein ( $10 \mu\text{g ml}^{-1}$ ) and Mu A protein ( $15 \mu\text{g ml}^{-1}$ ) and electrophoresed on 0.8% agarose gels as described<sup>4</sup>. Suboptimal concentrations of A were used deliberately, to avoid non-specific mobility shifts of all DNAs. Arrows point to type I complexes which migrate just above the supercoiled DNA position. Their formation was confirmed by electron microscopy (not shown). The absence or presence of A is denoted – or +. Multiple bands in the pRA170 sample occur because the sample has a significant fraction of dimeric molecules. **c**, Nicking at the ends of Mu. All lanes are as in **b**, except that A was at  $30 \mu\text{g ml}^{-1}$  and SDS was added to samples after the reaction<sup>4</sup>. Nicking at Mu ends occurs in the type I complex and results in relaxation of supercoiled molecules. (In previous *in vitro* studies, constructs similar to pRA166 (ref. 5) and pRA167 (ref. 4) have been used. Differences in the efficiencies of transpososome formation under the different assay conditions, however, preclude a direct comparison of these results with those reported here. In addition,



previous *in vivo* studies, particularly those with shorter end segments<sup>27</sup> have not strictly compared transposition frequencies of mini-Mu constructs that do or do not have the operator region.)



pRA174 respectively). Inverting the orientation of this region (pRA171 and pRA172), however, abolished synthesis, as did deleting it (pRA168). The plasmid pRA166, which has only 01, the strongest of the three operator sites and part of 02, partially stimulated end-synthesis. The behaviour of these plasmids *in vivo* (using a mating-out assay for transposition<sup>6</sup>) was consistent with their behavior *in vitro*, in that pRA168, pRA171, and pRA172 had ~100-fold lower transposition frequencies, and pRA166 had an ~15-fold lower frequency than pRA167. The plasmids pRA170 and pRA174 had similar transposition frequencies to pRA167 (data not shown). Predictably therefore, the mutant transposase  $\Delta$ 17 (reconstituted at its C-terminal end with wild-type sequence, but lacking domain I $\alpha$ ), was ~100-fold less active than wild-type in Mu transposition *in vivo*, irrespective of whether Mu DNA contained a normal operator region in its proper orientation (data not shown). These results suggest that the formation of an I $\alpha$ -operator complex is essential for efficient transposition.

Between the related, heteroimmune phages Mu and D108 (refs 1 and 7) the sequences essential for transposition are conserved, although the regulatory regions that span the repressor, operator, Ner and domain I $\alpha$  of A (ref. 8) are different. As the Mu A protein binds *att*L of D108 *in vitro*<sup>9</sup>, the observed specificities of Mu and D108 transposases for their respective left ends<sup>10</sup>, must involve sequences other than *att*L. This specificity may be conferred by the I $\alpha$  domains of the two transposases binding to their respective operators. Consistent with this idea, a hybrid Mu A protein with a D108 I $\alpha$  domain bound Mu *att* sites but failed to bind Mu operator (data not shown).

The finding that domain I $\alpha$  of the Mu A protein binds an enhancer-like sequence required for efficient transposition reveals a new aspect of the Mu transposition reaction. *Cis*-acting enhancer sequences, whose activities are independent of distance and orientation, have been found in two site-specific DNA inversion reactions<sup>11,12</sup>. A protein distinct from the recombinase binds to the enhancer, and possibly also to the recombinase, to correctly align the recombining partners in the synaptic complex<sup>13-15</sup>. Mu A combines transposition and enhancement activities in one polypeptide. Recently, the site-specific Int recombinase of phage  $\lambda$  has been shown to contain two autonomous DNA-binding domains<sup>16</sup>. The question arises therefore as to how Mu A interacts with the *att* and operator sites for synthesis of Mu ends. On linear DNA fragments, the A protein shows a lower affinity for the operator than for the *att* DNAs. On supercoiled DNA, however, especially in the presence of DNA-bending proteins like HU and/or IHF (the latter binds between 01 and 02 and reduces the negative supercoiling requirement for *in vitro* Mu transposition<sup>17</sup>; see Fig. 1a), the A protein may have a higher affinity for the operator<sup>18</sup>. The operator may act as a loading site for A protein, facilitating the interaction of A with one or both of the *att* sites. This two- or three-site interaction must be highly transient, as no DNA looping between the operator site and one or both of the *att* sites has yet been observed (ref. 4; R.H., unpublished data). Mu A protein bound at operator may interact simultaneously with both *att* sites to align the *att* sites for productive synthesis. Alternatively, interaction of operator-bound A with only one *att* end may activate A protein bound at that end to capture and synapse with the other end. Understanding the mechanism by which the pairwise DNA-binding capacity of Mu A is used for the synthesis of Mu ends within the transpososome will require further study. Finally, we add that K. Mizuuchi and co-workers have also observed the importance of the operator region in transposition. □

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## ATP-dependent assembly of double hexamers of SV40 T antigen at the viral origin of DNA replication

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SIMIAN virus 40 (SV40) replicates in nuclei of human and monkey cells. One viral protein, large tumour (T) antigen, is required for the initiation of DNA replication<sup>1,2</sup>. The development of *in vitro* replication systems which retain this property has facilitated the identification of the cellular components required for replication<sup>3-5</sup>. T antigen recognizes the pentanucleotide 5'-GAGGC-3' which is present in four copies<sup>6,7</sup> within the 64 base-pairs (bp) of the core origin<sup>8,9</sup>. In the presence of ATP it binds with increased affinity<sup>10,11</sup> forming a distinctive, bilobed structure visible in electron micrographs<sup>12</sup>. As a helicase<sup>13</sup>, it unwinds SV40 DNA bidirectionally from the origin<sup>14-16</sup>. We report here that *in vitro* and in the presence of ATP, T antigen assembles a double hexamer, centred on the core origin and extending beyond it by 12 bp in each direction. The assembly of this dodecamer initiates an untwisting of the duplex by 2-3 turns. In the absence of ATP, a tetrameric structure is the largest found at the core origin. In the absence of DNA, but in the presence of ATP or its non-hydrolysable analogues, T antigen assembles into hexamers. This suggests that ATP effects an allosteric change in the monomer. The change alters protein-protein interactions and allows the assembly of a double hexamer, which initiates replication at the core origin.

We used scanning transmission electron microscopy (STEM) to determine the sizes and masses of the nucleoprotein complexes formed after incubating T antigen with linearized pOR1

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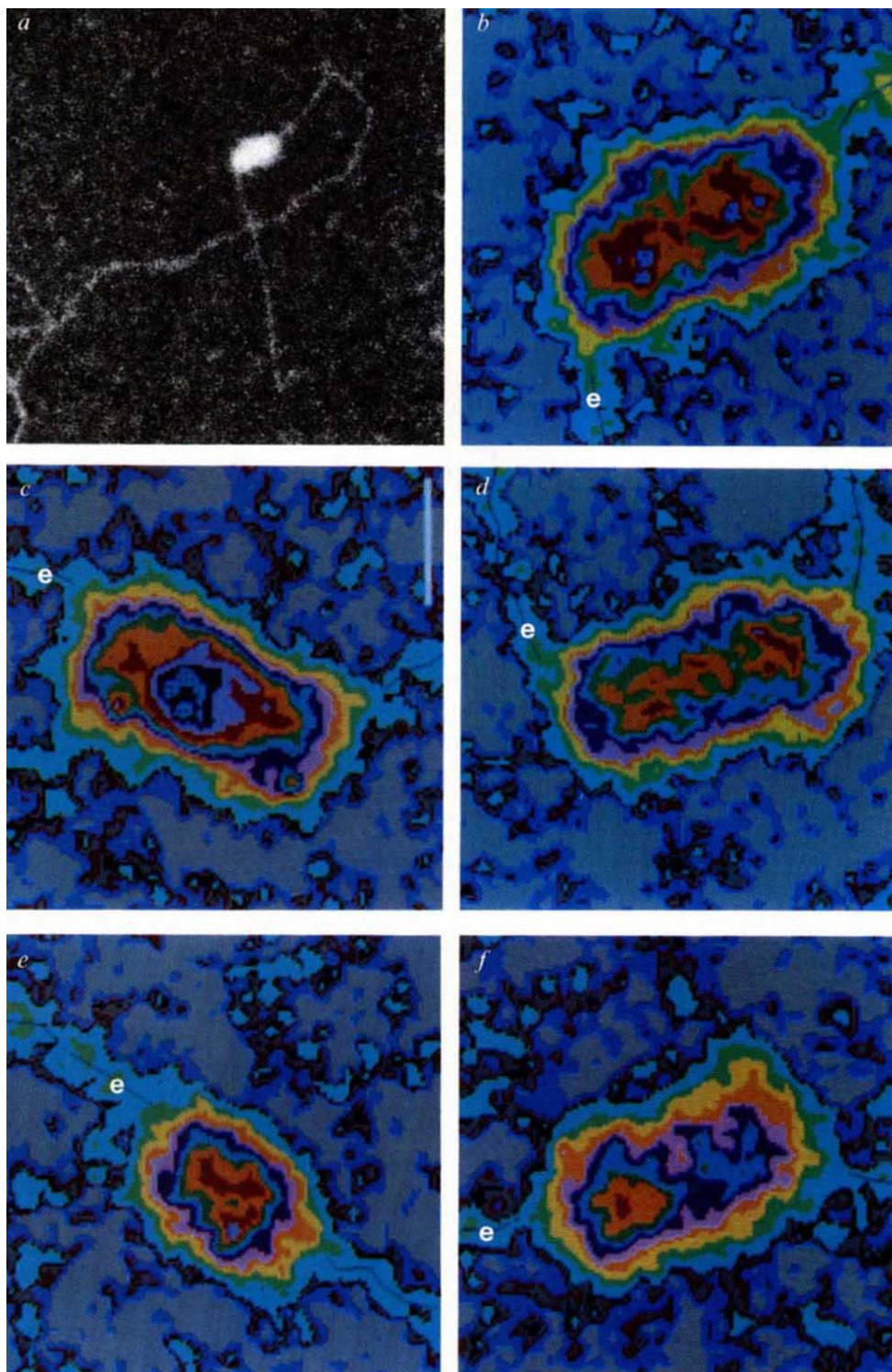


FIG. 2 T-antigen complexes bound at the SV40 core origin after incubation at 37 °C in the presence of 4 mM ATP. *a*, A low-magnification ( $1/4\ \mu\text{m} \times 1/4\ \mu\text{m}$ ) black and white digital image showing the overall binding geometry. *b*, A high magnification, colour-coded image of the 1,227K protein structure in *a*, representing scattered-electron counts for each  $5\ \text{\AA} \times 5\ \text{\AA}$  element; the counts are proportional to mass thickness defined as  $\text{K nm}^{-2}$ . For each colour change, mass thickness increases by a 0.6 nm step of compact protein (material of density  $1.33\ \text{g cm}^{-3}$  (see ref. 19)). The black lines on either side of the protein show the DNA path; *e* marks the early-transcription side. The white bar is 10 nm in length. *c*, 1,187K structure. *d*, 1,121K structure. *e*, 556K structure. *f*, 850K structure.

**METHODS.** T antigen was purified by a modification of the procedure of Dixon and Nathans<sup>35</sup>. A lysate of cs1085-infected Cos-1 cells was prepared and applied to an immuno-affinity column. The column was washed with a solution containing 50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1 mM EDTA, 10% glycerol and 1% NP-40 but lacking dithiothreitol (DTT). The column was then washed with a similar buffer, lacking both NP-40 and DTT. T antigen was eluted from the column with a solution of 50% ethylene glycol, 20 mM Tris-HCl, pH 8.5, 500 mM NaCl, 1 mM EDTA and 10% glycerol. Fractions containing T-antigen DNA-binding activity were pooled and dialysed against storage buffer (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 1 mM DTT and 50% glycerol) and stored unfrozen at  $-20\ ^\circ\text{C}$ . ~95% of the protein was T antigen. Reaction conditions and grid preparation were modifications of previous methods<sup>12,16,18</sup>. Standard reaction mixtures (20  $\mu\text{l}$ ) contained 40 mM creatine phosphate (diTris salt, pH 7.7), 7 mM  $\text{MgCl}_2$ , 0.5 mM DTT, 0.7  $\mu\text{g}$  creatine kinase, 4 mM ATP, 0.5  $\mu\text{g}$  of plasmid pOR1 DNA (Fig. 1) and 0.4  $\mu\text{g}$  T antigen and were incubated for 1 h at  $0\ ^\circ\text{C}$  or  $37\ ^\circ\text{C}$ . In these experiments, the T antigen:DNA mole ratio was 20 to 1. DNA, ATP and creatine kinase were omitted where indicated. Glutaraldehyde was added to 0.1%, with incubation for an additional 15 min. Reaction mixtures were passed over 0.3 ml Sepharose 4B columns equilibrated with 40 mM Hepes/KOH (pH 7.6) and 11 mM magnesium acetate. Peak column fractions were adsorbed to thin carbon foil treated with polylysine. Grids bearing the foil were immersed in liquid  $\text{N}_2$  and dried by sublimation at low temperature and a pressure of  $10^{-6}$  torr or less. In the STEM, nucleoprotein structures were examined at  $-150\ ^\circ\text{C}$  with beam exposures

DNA (Fig. 1). In STEM, molecular images are constructed from the intensities of scattered electrons, measured at positions separated by  $5\ \text{\AA} \times 5\ \text{\AA}$  in a  $512 \times 512$  matrix. The method depends on the known proportionality between scattered intensity and mass for the atoms comprising biological macromolecules<sup>17</sup>. A resolution approaching  $20\ \text{\AA}$  is obtained, which is sufficient to observe substructure in large molecules<sup>18,19</sup>.

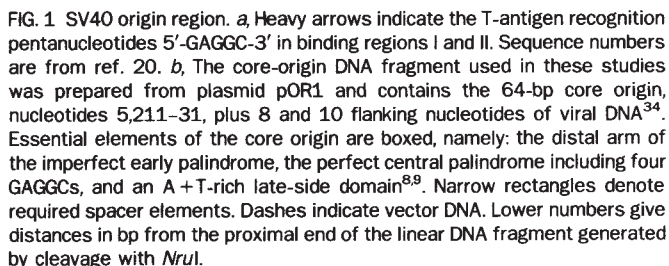
Figure 2 shows examples of the structures found at the SV40 core origin, after incubation with T antigen at  $37\ ^\circ\text{C}$  in the



of  $10\text{--}50\ \text{electrons}\ \text{\AA}^{-2}$ . The principles of STEM mass measurement and data analysis have been described<sup>17,18,19</sup>. Note that the total mass of a macromolecule, as measured by electron scattering, is independent of its orientation on the foil.

presence of ATP. One is shown in a low-magnification, high contrast micrograph (Fig. 2*a*), resembling those observed by conventional microscopy<sup>12</sup>, and also at high magnification where intensities from individual probe positions can be resolved (Fig. 2*b*). In one of three replicate incubations that gave similar results, eight large structures were observed whose dimensions both parallel and perpendicular to the DNA were comparable, and whose relative molecular masses ( $M_r$ ) were in the range 1,100,000–1,200,000 (1,100K–1,200K) (Fig. 3*a*). Computer



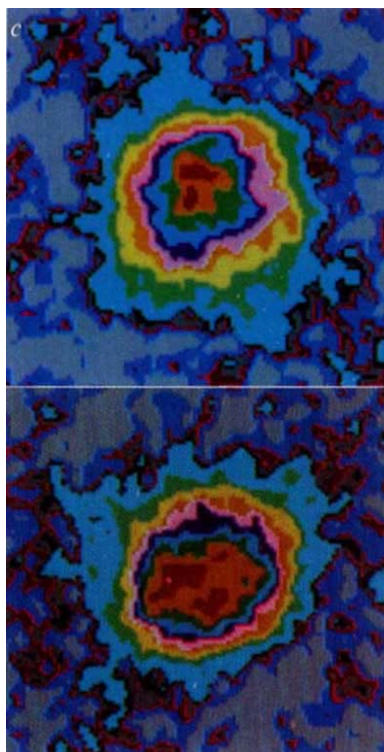


complexes visualized peaked at  $1,156 \pm 47\text{K}$  ( $n = 8$ ) and had a standard deviation of less than 5% (Fig. 3a), consistent with the known precision of mass measurement by STEM. The maximal (1,156 K) structure was centred on the origin and extended along  $89 \pm 5$  bp, suggesting that this assembly is responsible for the origin-centred, DNase-I-protected fragment length of 80–84 bp (refs 10 and 11).

Of the complexes visualized, ~5% were larger than the 1,156 K structure (data not shown). These were characterized by a complete 1,156 K assembly on the early side of the core origin and a partial assembly at the core origin itself. The structure of the complexes whose  $M_r$  values were less than that of 1,156 K structure fell within the outline of the maximal structure, indicating that they were partially formed variants of this complex (see Fig.2e,f). Essentially all the nucleoprotein binding involved the core origin.

The mean mass (1,156K) associated with the maximal complex is 14.1 times greater than the  $M_r$  of 82K predicted for an unmodified T-antigen monomer from the SV40 DNA sequence<sup>20</sup>. As with most STEM applications, however, this mass was measured relative to the mass per unit length of the 39,300K tobacco mosaic virus (TMV)<sup>21</sup>. To calibrate the measurement accurately enough to determine the precise number of T-antigen monomers present in the complex, mass measurements were carried out in the absence of DNA.

The incubation of T antigen at 37 °C in the absence of DNA but in the presence of ATP resulted in the formation of oligomers whose mean masses were 1, 2, 3 and 6 times that of the lowest-mass peak (Fig. 4a). Without ATP, peaks corresponding to oligomers of mass 1, 2, 3 and 4 times that of the lowest were obtained (Fig. 4b). On the basis of these results the apparent monomer  $M_r$ , measured relative to TMV, was determined to be  $98 \pm 3K$  (Fig. 4 inset). We note, however, that the difference between this value and that predicted from DNA sequence need not necessarily be due to an imprecision in the TMV calibration, but could result from an additional mass component associated with each monomer.



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The proportion of T antigen incorporated into the hexamer (Fig. 4c) formed in the presence, but not the absence, of ATP varied from 30–80% in individual experiments. Further evidence for its formation was obtained by glycerol-gradient centrifugation: in the absence of ATP a single band of T antigen was detected whereas in the presence of ATP a band whose sedimentation rate was consistent with the formation of a hexamer was observed in addition to a monomer or monomer-dimer band (data not shown). The hexamer band contained more than 50% of the protein. The fact that the protein oligomers of intermediate sizes detected by STEM were not evident in the glycerol-gradient profiles may be attributed to the presence of glutaraldehyde in the incubations for microscopy, which can stabilize these oligomers<sup>12</sup>. An additional rapidly sedimenting band of T-antigen in gradients lacking ATP has also been reported<sup>22–25</sup>. This may correspond to hexamers formed *in vivo*, an interpretation supported by our observation that hexamers assembled in the presence of ATP remain stable on sedimentation through glycerol gradients in the absence of ATP (unpublished observations).

After precise calibration, the  $M_r$  of the 1,156 K complex formed at the core origin was determined to be  $11.8 \pm 0.4$  times that of the monomeric protein. The probability that the complex comprises 12 rather than 11 or 13 proteins is therefore greater than 95%. Furthermore, given the bidirectional nature of unwinding and DNA replication, the symmetry of the central palindrome and the symmetry of the structures observed, T antigen is probably utilized in equal numbers on the two sides of the origin. A 10-mer or 14-mer, the closest even alternatives to a 12-mer, are excluded by 5 standard deviations. The bilobed nature of some of the maximal structures (Fig. 2b), in which the individual lobes resemble the hexameric structures that assemble in the absence of DNA (Fig. 4c), indicates that the nucleoprotein complex is a double hexamer of T antigen, with each hexamer bound to one half of the palindrome at the centre of the SV40 core origin.

Neither replication nor unwinding of DNA by T antigen occurs at 0 °C, with or without ATP<sup>14,26</sup>. In search of a structural correlate, we examined the binding of T antigen to core-origin DNA at 0 °C in the presence of ATP (Fig. 3b). The binding remained entirely specific for the origin DNA sequences, but the largest structure was a 9-mer and not a 12-mer. The mass distribution for each complex was within the normal outline of a 12-mer and 3–6 monomer units were present within each half of that outline. We conclude therefore that assembly of the complete 12-mer complex of T antigen does not occur at 0 °C.

Little T-antigen binding occurs at the core origin at 37 °C in the absence of ATP<sup>10–12</sup>. We therefore examined the structures formed in the absence of ATP at 0 °C. Under these conditions DNase I footprints spanned the entire 64-bp stretch<sup>10,11</sup>. These structures proved to be mainly of tetramer mass, with a few complexes of trimer mass (data not shown). This result agrees with our earlier investigation which showed that a maximum of four monomers corresponding to the number of recognition sites bound in the absence of ATP<sup>18</sup>.

The formation of new quaternary structures suggests that ATP alters the conformation of the monomer to allow new protein-protein interactions. Other studies have shown that non-hydrolyzable analogues of ATP act to increase DNase I protection<sup>10,11</sup> and also lead to hexamer formation in the absence of DNA (unpublished observations). The induced monomer-monomer interactions do not therefore require ATP hydrolysis. Allosteric effects of ATP and GTP binding have been proposed as the basis of an enhanced DNA-binding capacity in a number of proteins, including the helicases *dnaB* of *E. coli* and gene 41 protein of bacteriophage T4 (refs 27–29). Interestingly, the *dnaB* protein and the *E. coli rho* protein exist as native hexamers with helicase activity<sup>30–32</sup>.

The interaction of the ATP-altered, T-antigen monomer with recognition pentanucleotides may not be substantially different

from that of the unaltered monomer. This is implied by the equivalent pentanucleotide protection pattern provided by T-antigen binding in reactions with and without ATP<sup>6,7,33</sup>. We suggest that monomer binding at the two pentanucleotides in each half of the central palindrome serves to nucleate hexamer formation. The double hexamer thus formed is envisioned as two regular, hexagonal rings surrounding core-origin DNA. In accord with this model, the length of DNA containing the complex remains unchanged<sup>12</sup> and DNase I protection virtually blanks out 84 bp<sup>10,11</sup> as one would expect if the DNA is enveloped by protein.

The double hexamer probably has as one function the local denaturation of DNA. The favourable free energy change for the formation of the double hexamer must therefore be larger than the unfavourable change associated with the concomitant changes in DNA conformation. The hexamers may be regular, with any major conformational irregularities being assumed by the DNA: diametrically related members of each hexagonal ring can interact with adjacent pentanucleotides located 6 bp apart if the net free energy change allows an untwisting of the helix angle from 205° to 180°. The difficulty in forming a 12-mer at 0 °C has a natural interpretation: a 9-mer may assemble without severe steric problems but a 12-mer may require local denaturation or other conformational changes in the DNA, that are not thermodynamically feasible at 0 °C.

Recent observations show that T antigen bound at the origin untwists the DNA helix by 2–3 turns in the absence of single-stranded DNA-binding protein. This untwisting is not observed in the absence of ATP or at temperatures below 15 °C (F.D. and

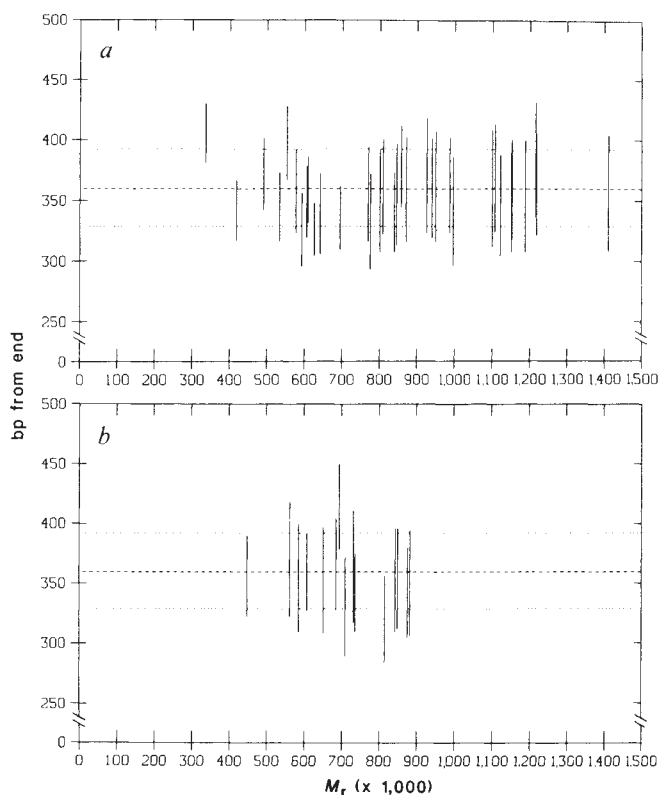


FIG. 3 T-antigen binding events occurring in the presence of ATP on core-origin DNA after incubation at a, 37 °C or b, 0 °C. Each structure is described by a vertical line, displaced horizontally in proportion to total mass of protein bound and vertically according to the location of the complex on the core origin fragment. The dotted horizontal lines indicate the boundaries of the SV40 core origin, the dashed line the centre of the 27-bp palindrome. In a,  $n=34$  and in b,  $n=15$ . The molecules in each panel are from one incubation. All complexes bound to reasonably well-spread DNA were measured.



J.H., unpublished observations). Thus, it seems that 2–3 turns of untwisting occurs on completion of the double hexamer. Borowiec and Hurwitz<sup>33</sup> have observed localized melting and helical distortion in the early- and late-side elements of the core origin, respectively. The full 2–3 turns of untwisting are probably due to more complete melting of the double helix in the early and late elements which together comprise about 3 turns.

Whether the individual hexamer or the dodecamer constitutes the basic helicase will be determined by studying the structures present at unwinding forks. The first alternative would require fission of the double hexamer and the second, assembly of two dodecamers. As noted above, paired dodecamers have been observed. □

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## Identification of $\gamma$ -tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*

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**MICROTUBULES**, which are essential for mitosis and many other cytoskeletal functions, are composed primarily of  $\alpha$ - and  $\beta$ -tubulin. The properties of microtubules are due, in part, to proteins other than tubulins that are part of, or interact with, microtubules and the identification and characterization of such proteins is important to understanding how microtubules function. Analyses of mutations at the *mipA* (microtubule interacting protein) locus of *Aspergillus nidulans* have suggested that the product of *mipA* interacts specifically, probably physically, with  $\beta$ -tubulin *in vivo* and is involved in microtubule function<sup>1</sup>. We have cloned and sequenced the wild-type *mipA* gene as well as complementary DNA copies of its messenger RNA. Comparisons of the predicted product of *mipA* with tubulins from diverse organisms reveal that *mipA* is a previously undiscovered member of the tubulin superfamily of genes; the only member yet discovered that does not encode  $\alpha$ - or  $\beta$ -tubulin. We propose that the product of *mipA* be called  $\gamma$ -tubulin.

Three mutant *mipA* alleles were isolated as extragenic suppressors of *benA33*, a heat-sensitive (*hs*<sup>−</sup>)  $\beta$ -tubulin mutation<sup>1,2,3</sup>. The *benA* gene encodes the major  $\beta$ -tubulin expressed in hyphae<sup>4</sup> and this  $\beta$ -tubulin is essential to mitosis and nuclear migration<sup>2,5</sup>. Although the mutant *mipA* alleles confer cold-sensitive (*cs*<sup>−</sup>) inhibition of growth in strains carrying certain *hs*<sup>−</sup> *benA* alleles (but not the wild-type *benA* allele (*benA*<sup>+</sup>)), the inhibition is insufficient to allow the selection of wild-type *mipA* (*mipA*<sup>+</sup>) transformants. The *mipA* mutations map 0.22–0.33 cM to the left of the *riboB* locus<sup>1</sup>, however, and this linkage allowed us to clone *mipA*<sup>+</sup> by chromosome walking from the previously cloned *riboB* gene<sup>6</sup>.

By co-transformation experiments using *A. nidulans* strain LO519 (Fig. 1), we were able to identify a 4.8-kilobase (kb)

*EcoRI* fragment (fragment 3) that complements *mipA1*. Additional co-transformation experiments (not shown) indicated that a 2.8-kb *HindIII-SacI* fragment (HS2.8) within fragment 3 complemented *mipA1*. We constructed a plasmid, pLO9, carrying HS2.8 and a 2.3-kb *NdeI-EcoRI* fragment containing the wild-type *pyrG* gene<sup>7</sup> (*pyrG*<sup>+</sup>), both cloned into pUC19 (ref. 8). We transformed LO519 with pLO9 and chose 14 *pyrG*<sup>+</sup> transformants. Thirteen of these were found to have the wild-type *mipA* (*mipA*<sup>+</sup>) phenotype. Southern hybridizations (not shown) revealed that in the 13 *mipA*<sup>+</sup> transformants, one or more copies of pLO9 had integrated into the genome. In the single transformant with the mutant *mipA* phenotype, no plasmid sequences were present, indicating that the wild-type *pyrG* allele had replaced the mutant allele by gene conversion. In two *mipA*<sup>+</sup> transformants a single copy of pLO9 had integrated at the *pyrG* locus. Since HS2.8 is closely linked in the genome to *riboB*, as is *mipA*, and complements *mipA1* in *cis* or in *trans* (that is, when integrated at *pyrG*), we conclude that HS2.8 carries *mipA*<sup>+</sup>.

We sequenced HS2.8 and all or portions of six cDNAs that hybridized to HS2.8. These results revealed that HS2.8 contains one complete gene and a second 150 amino-acid open reading frame (ORF). Southern and northern hybridizations and sequencing of genomic and cDNA clones (Fig. 2 and additional data not shown) revealed that the 150 amino-acid ORF is approximately half (the 3' half) of the coding region of a gene, the 5' half of which is on an adjacent fragment. Because the 3' half of a gene, lacking a promoter, is unlikely to function *in trans*, we conclude that the complete gene on HS2.8 is *mipA*<sup>+</sup>.

The sequence of *mipA*<sup>+</sup> is shown in Fig. 2. The *mipA* cDNA contains a single, long ORF encoding 454 amino acids. High stringency Southern (not shown) and northern hybridizations (Fig. 3) revealed that *mipA* is a single copy gene that produces a moderately abundant 1.5-kb mRNA. Although we have not yet demonstrated that the *mipA* mRNA is translated into a protein, the presence of a 454 amino-acid ORF in the cDNA, the fact that the mutant *mipA* alleles cause cold sensitivity in combination with certain *benA* alleles and the fact that the cold sensitivity is suppressed by a single copy of the *mipA*<sup>+</sup> gene argue strongly that the functional product of the *mipA* gene is a protein, the protein encoded by the 454 amino-acid ORF.

Examination of the amino-acid sequence of the *mipA* gene product (MAGP) revealed a substantial similarity to  $\alpha$ - and  $\beta$ -tubulin. Figure 4 shows a comparison of MAGP and pig brain  $\beta$ -tubulin<sup>9</sup>. The two sequences align readily with only a few

small gaps required to maintain the alignment. Overall, 151/454 (33.3%) of the amino acids of MAGP are identical to the corresponding  $\beta$ -tubulin amino acids. Statistical analysis (Fig. 4) revealed that the chances of unrelated proteins of the same size and amino-acid composition showing such identity are less than  $7.6 \times 10^{-24}$ .

Comparisons of MAGP with 29 additional  $\alpha$ - and  $\beta$ -tubulin sequences from mammals, birds, insects, algae, protozoans and fungi (all of the complete tubulin sequences in the Protein Information Resource release 17 as well as the two *A. nidulans*  $\beta$ -tubulins<sup>10</sup> and  $\alpha$ -tubulins (Drs P. Doshi and N. R. Morris, unpublished results)) yielded similar results (32.3–35.2% identity for MAGP/ $\beta$ -tubulin comparisons and 28.9–31.7% identity for MAGP/ $\alpha$ -tubulin comparisons). All matches were highly significant ( $>29$  standard deviations above random). Several regions are remarkably conserved among MAGP and all the  $\alpha$ - and  $\beta$ -tubulins examined. These include regions thought to be involved in GTP binding<sup>11–13</sup> (MAGP amino acids 67–70, 143–148, 205–208).

All  $\alpha$ -tubulins reported to date share  $>62\%$  identity, all  $\beta$ -tubulins share  $>63\%$  identity and  $\alpha$ - and  $\beta$ -tubulins share 36–42% identity with each other<sup>14</sup>. MAGP is thus not  $\alpha$ - or  $\beta$ -tubulin but is nearly as similar to  $\alpha$ - and  $\beta$ -tubulins as they are to each other. Dayhoff *et al.*<sup>15</sup> have proposed that proteins that have  $>50\%$  identity be considered members of the same family and proteins with statistically significant similarities (probability of similarity occurring by chance  $<10^{-6}$ ) be considered part of the same superfamily. By this reasonable convention, all  $\beta$ -tubulins are members of one family, all  $\alpha$ -tubulins

are members of a second family and  $\alpha$ - and  $\beta$ -tubulins are members of the tubulin superfamily. MAGP clearly is a member of the tubulin superfamily and we propose that it be called  $\gamma$ -tubulin.

Sequence comparisons also give clues to the origin of  $\gamma$ -tubulin. There are three obvious possibilities. The first, that it was formed recently by the fusion of a tubulin gene and a non-tubulin gene, is precluded by the existence of regions of

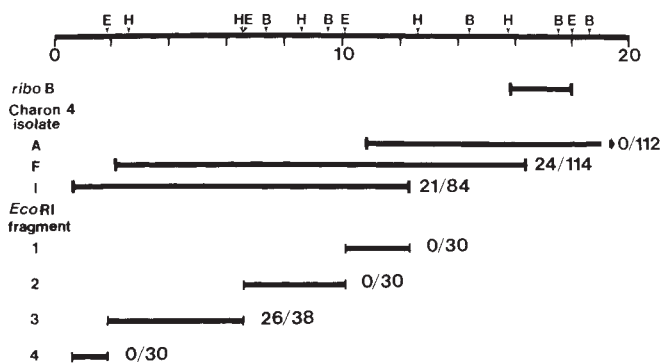


FIG. 1 Identification of the wild-type (wt) *mipA* gene. At the top is a restriction map of the region of linkage group VIII containing and extending to the left (centromere proximal side) of the *riboB* gene. Only the *Bam*HI (B), *Eco*RI (E) and *Hind*III (H) sites are shown and the scale is in kb.

**METHODS.** The map was constructed by restriction-site mapping Charon 4 isolates carrying inserts A, F and I, identified by chromosome 'walking' from the *riboB* gene using a library of wt *A. nidulans* sequences<sup>17</sup>. Transformation of protoplasts of *A. nidulans* with a mixture of two types of DNA results in a fraction of the protoplasts that are transformed with one type being co-transformed with the second (ref. 18 and S. Osmani, personal communication). To identify Charon 4 isolates carrying the wt *mipA* gene we constructed a strain (LO519) carrying *mipA1*, *benA33* and *pyrG89*. In combination, *mipA1* and *benA33* confer cold sensitivity<sup>1</sup> and *pyrG89* is a mutation in the orotidine-5'-phosphate decarboxylase gene that prevents growth in the absence of uridine or uracil<sup>19</sup>. Protoplasts of LO519 were transformed with a plasmid carrying the wt *pyrG* gene (*pyrG*<sup>+</sup>) and restriction digests of the Charon 4 isolates, *pyrG*<sup>+</sup> transformants were selected and tested for complementation of *mipA1*. Wild-type *mipA* (*mipA*<sup>+</sup> transformants) are not cold-sensitive. The results of these experiments are expressed as fractions to the right of each Charon 4 insert. Thus, of 114 *pyrG*<sup>+</sup> transformants tested from a *pyrG*<sup>+</sup>/isolate F co-transformation, 24 were *mipA*<sup>+</sup>. Isolates F and I thus contain the wt *mipA* gene. Co-transformation experiments were subsequently carried out with *Eco*RI fragments of the insert in isolate I. (The *Eco*RI sites at the end of the inserts are from linkers and are not present in the genome.) Again the results are expressed as a fraction to the right of each fragment. Fragment 3 thus contains the wt *mipA* gene.

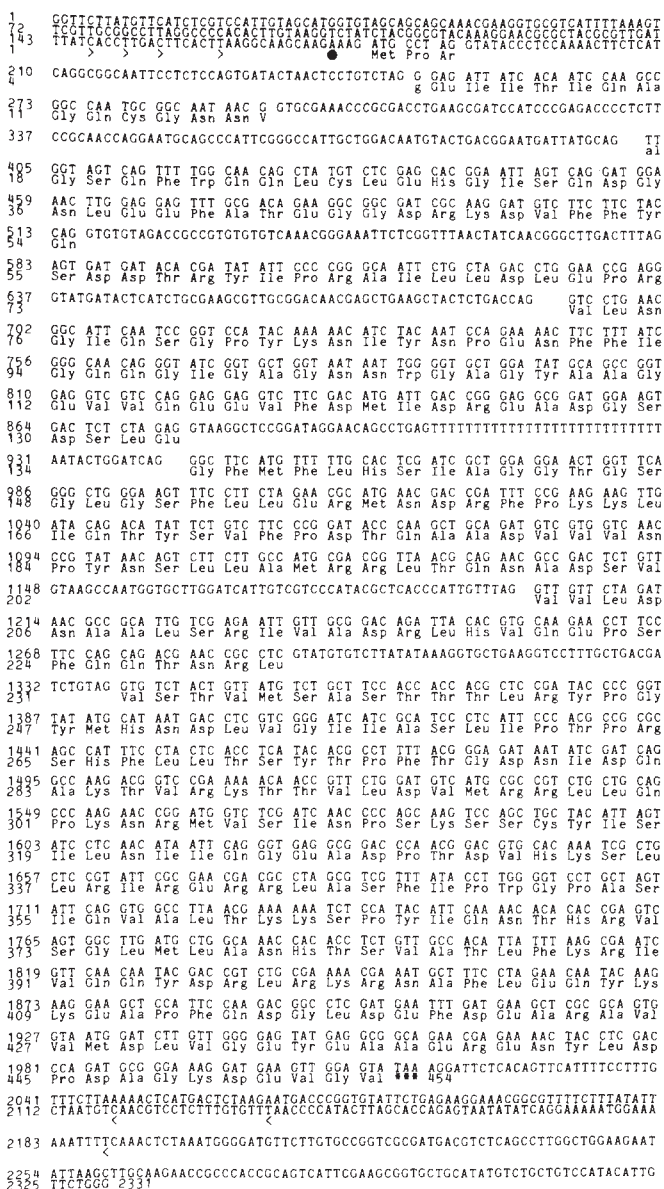


FIG. 2 The sequence of the wt *mipA* gene. The genomic clone was sequenced completely on both strands using dideoxy sequencing from custom-synthesized primers. Transcription start sites ( $>$ ) were determined by S1-nuclease-mapping<sup>10</sup> using a uniformly labelled probe synthesized by extending a custom synthesized primer that anneals to a region in intron 1. The amino acid sequence is shown below the nucleotide sequence. It was determined as follows. A cDNA from a library constructed by Osmani *et al.*<sup>20</sup> was sequenced completely on both strands and found to contain a 451 amino-acid ORF without an initiation codon. To determine the sequence of the 5' end of the ORF, additional cDNAs were isolated and the ends sequenced. The 5' end of the cDNA that terminated nearest the 5' end of the mRNA is designated  $\bullet$ . The 3' ends of the cDNAs sequenced were the nucleotides designated  $<$ . The predicted product of *mipA* has a relative molecular mass of 50,825 and an isoelectric pH of 5.55. The gene has seven introns and, in common with several other *A. nidulans* genes,<sup>7,10</sup> lacks a typical TATA box or consensus polyadenylation signal.



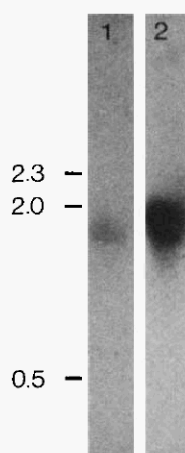


FIG. 3 *MipA* and *benA* northern hybridizations. Lane 1 contains 10  $\mu$ g of *A. nidulans* polyA RNA (isolated by a minor modification of the method of Osmani *et al.*<sup>21</sup>) fractionated on a 1% agarose gel containing 3.1% formaldehyde and probed with 10 ng ( $10^7$  c.p.m.) of a 2.1-kb DNA fragment containing the *mipA* gene and no portions of adjacent genes. Lane 2 contains 5  $\mu$ g of the same RNA probed with 10 ng ( $10^7$  c.p.m.) of a 2.7-kb fragment carrying the *benA* gene and no portions of adjacent genes. The fragment sizes indicated at the left are from single-stranded DNA fragments from a *HindIII* digest of bacteriophage  $\lambda$ . The *benA* mRNA is slightly longer than the *mipA* mRNA because it has a longer 5' untranslated region<sup>10</sup>. Hybridization was for 12 h in  $6\times$ SSC, 0.25% non-fat dry milk, 50% formamide at 42 °C and washing was at 70 °C (two 20-min washes in  $2\times$ SSC, 0.01% SDS and two 20 min washes in  $0.2\times$ SSC, 0.01% SDS). Radioautography and photography of the autoradiograms for the two samples was identical. Although radioautography of northern hybridizations is not a precise method for quantitating mRNA levels, densitometry scans of the autoradiograms suggest that the *mipA* mRNA level may be  $\sim 5\%$  of the level of the abundant *benA* mRNA.

|             |       |       |       |       |       |       |       |       |       |       |       |     |
|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| <i>mipA</i> | MPREI | ITIQ  | GQCCN | NVQSG | FQQQL | CLEHG | ISQDC | NLEEF | ATEGG | DRKDV | FFYQS | 55  |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | MREI  | VHIQA | GQCCN | QIGAK | FWEWI | SDEHG | IDPTG | SYRGD | SDQLQ | ERINV | YYNEA | 54  |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | DDTRY | IPRAI | LDLDE | PRVLN | GIQSG | PKYNI | YNFEN | FFIQG | QGIGA | GNNGW | AG-YA | 109 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | AGNKY | VPRAI | LDLDE | PCTMD | SVRSG | PGFQI | FRFDN | VFVQG | SG--A | GNNGW | KCHYT | 107 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | AGEVV | QEEVF | DMIDR | EADGS | DSLEQ | FMFLH | SIAGG | TCGSL | GSFLF | ERMND | RFPKK | 164 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | EGAEI | VDSVL | DVVRK | ESESC | DCLQG | FQLTH | SLGGC | TCGSM | CTLLI | SKIRE | EYPDR | 162 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | LIQTY | SVFPD | TQAAD | VVVNF | YNSLL | AMRRL | TQNAD | SVVVL | DNAAL | SRIVA | DRLHV | 219 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | IMNFT | SVVPS | PKVSD | TVVEP | YNATL | SVHQL | VENTD | ETCYI | DNEAL | YDICF | RTLKL | 217 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | QEFSF | QQTNR | LVSTV | MSAST | ITLRY | PGYMH | NDLVG | IIASL | IPTPR | SHFLF | TSYTP | 274 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | TIPTY | GDNLH | LVSAT | MSGVT | TCLRQ | PGQLN | ADLRK | LAVNM | VYFPR | LHFEM | PGFAP | 272 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | FTGDN | IDQAK | TVRKT | TVLDV | MRRLL | QPKNR | MVSIN | PSKSS | CYISI | LNIIQ | GEADP | 329 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | LTSRG | SQQYR | AL--- | TVPEL | TQQMF | DAKNN | MAACD | FRHGR | -YLTV | AAVFR | GRMSH | 323 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | TDVHK | SLLRI | RERRL | ASFIP | WGPAS | IQVAL | TKKSP | YIQNT | HRVSG | LMLAN | HTSVA | 384 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | KEYDE | QMLNV | QKNKS | SYFVE | WIPNN | VKTAV | CDIPP | ---RG | LKMSA | TFIGN | STAIK | 375 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | TLFKR | IVQQY | DRLRK | RNAFL | EQYKK | EAPFP | DGLDE | FDEAR | AVVMD | LVGEY | EAAER | 439 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>pigβ</i> | ELFKR | ISEQF | TAMFR | RKAFL | HWYTG | EG--H | DEM-E | FTEAE | SNMND | LVSEY | QQYQD | 427 |
|             | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   | ***   |     |
| <i>mipA</i> | ENYLD | PDAGK | DEVGV |       |       |       |       |       |       |       |       | 454 |
|             | ***   | ***   | ***   |       |       |       |       |       |       |       |       |     |
| <i>pigβ</i> | ATADE | QGEFE | EEGEE | DEA   |       |       |       |       |       |       |       | 445 |
|             | ***   | ***   | ***   | ***   |       |       |       |       |       |       |       |     |

FIG. 4 A comparison of the amino-acid sequences of the *mipA* gene product and pig brain  $\beta$ -tubulin<sup>9</sup>. Double asterisks are between identical amino acids and single asterisks are between conservative amino-acid substitutions following the convention of Dayhoff *et al.*<sup>22</sup>. We aligned the sequences using programs supplied by Dr M. Murata (University of Georgia) that use the algorithm of Murata<sup>23</sup> and the weighting system of Dayhoff *et al.*<sup>24</sup>. The significance of the MAGP, pig brain  $\beta$ -tubulin match was evaluated using the Murata programs as follows. First, the amino-acid sequences of the two proteins were aligned and an alignment score computed. The two sequences were then scrambled separately and an alignment score was computed for the two scrambled sequences. The scrambling and alignment process was carried out 100 times and a mean and standard deviation were determined for the alignment scores of the scrambled sequences. The alignment score for MAGP and pig brain  $\beta$ -tubulin sequences was higher than the mean alignment score for the scrambled sequences by 36.7 standard deviations. As the probability of an alignment score of two unrelated proteins being 10 standard deviations above the mean alignment score for the scrambled sequences of the two proteins is  $7.6 \times 10^{-24}$  (ref. 15), the similarity of the *mipA* gene product to pig brain  $\beta$ -tubulin is highly significant.

identity between  $\gamma$ -tubulin and  $\alpha$ - and  $\beta$ -tubulins along the entire length of the molecules (except for the extreme C-terminal region which is highly divergent in all tubulins; see Fig. 4). A second possibility, that it has diverged recently from an  $\alpha$ - or  $\beta$ -tubulin gene and evolved rapidly, predicts that  $\gamma$ -tubulin should be most similar to a tubulin in *A. nidulans* or closely related species. In fact  $\gamma$ -tubulin is no more similar to *A. nidulans* tubulins than tubulins from other organisms and the greatest identity we found (35.2%) was with *Drosophila melanogaster*  $\beta$ -3 tubulin<sup>16</sup>. In addition, in certain regions ( $\gamma$ -tubulin amino acids 26–31, 51–52, 153–156, 228–230, 376–384) there is greater identity between  $\gamma$ - and  $\alpha$ -tubulins than between  $\alpha$ - and  $\beta$ -tubulins and in other regions (amino acids 386–390, 429–434) there is greater identity between  $\gamma$ - and  $\beta$ -tubulins than between  $\alpha$ - and  $\beta$ -tubulins. If  $\gamma$ -tubulin had diverged recently from a  $\beta$ -tubulin, for example, it should be more similar to  $\beta$ -tubulin than  $\alpha$ -tubulin throughout the molecule. These data, in sum,

are inconsistent with a recent origin of  $\gamma$ -tubulin and the simplest hypothesis consistent with these data is that  $\gamma$ -tubulin diverged from a tubulin ( $\alpha$ ,  $\beta$  or ancestral) at about the time of the  $\alpha/\beta$ -tubulin divergence, near the time of the first eukaryotes. Since there is no obvious reason that  $\gamma$ -tubulin should have been retained only in *A. nidulans*, it is probable that it (and perhaps other, undiscovered tubulins), will be present in many, perhaps all, eukaryotic organisms. □

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# Protein crystal dynamics studied by time-resolved analysis of X-ray diffuse scattering

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It has been known for many years that information about the dynamics of molecules in crystals can be obtained from diffusely scattered radiation<sup>1-3</sup>. Intermolecular motions coupled on a large scale generate diffuse spots centred on the Bragg peaks, whereas locally coupled intramolecular motions produce a diffuse background. Although information about protein dynamics has recently been derived from diffusely scattered radiation<sup>4-6</sup>, molecular motions could not be distinguished from static disorder in these experiments, because of a lack of time resolution. Provided the energy of the radiation is well defined, however, time resolution can be obtained from an energy analysis of the scattered radiation. We report here the use of Mössbauer radiation, the energy of which is characteristically well defined, to probe the molecular dynamics of a myoglobin crystal. Information about molecular motions is extracted from the ratio of elastically to inelastically scattered photons, which by using Mössbauer radiation can be precisely determined<sup>7</sup>. At room temperature we obtain a root-mean-squared amplitude for intermolecular motions of 0.13 Å.

The energies of 'elastic'  $\gamma$ -rays, emitted without recoil by an <sup>57</sup>Fe source, fall within a narrow band of width  $4.66 \times 10^{-9}$  eV. The  $\gamma$ -rays incident on a protein crystal are scattered by the electrons of the sample. Inelastic scattering occurs as a result of thermal molecular motions, whereas static disorder does not give rise to inelastic processes. To determine the relative intensities of elastically and inelastically scattered radiation we used a <sup>57</sup>Fe Mössbauer absorber as energy analyser in the incident and the scattered  $\gamma$ -beam, respectively. By moving the source relative to this analyser the energy band of the <sup>57</sup>Fe radiation can be shifted in or out of resonance absorption. The number of quanta removed from the beam at resonance condition measures the content of 'elastic'  $\gamma$ -rays (for details see Fig. 1).

To overcome intensity problems and avoid interferences, only strong reflections were studied. Figure 2 shows the total intensity of scattered radiation together with the intensity of inelastically scattered radiation measured for the 16 2 1 reflection, which has a Bragg spacing of 3.63 Å. For the inelastic scattering, a pedestal to Compton and slowly varying diffuse scattering was determined at  $\theta = 6.3^\circ$  and  $\theta = 7.7^\circ$ . The intensity of inelastically scattered radiation which peaked at the reciprocal node accounted for 5% of the total intensity from the reflection.

The information about myoglobin dynamics was obtained from the inelastic diffuse scattering as this results from energy transfer with thermal molecular motions. In practice, distinguishing between 'elastic' and 'inelastic' diffuse scattering depends on the resolution of the incident radiation. With <sup>57</sup>Fe Mössbauer radiation, energy transfers of the order of  $10^{-9}$  eV can be resolved and radiation resulting from larger energy transfers yields the time dependence of the thermal motions. Thus, motions with a characteristic time greater than 100 ns cannot be discriminated and are treated as static disorder.

The inelastic scattering due to intermolecular motions occurs near to reflections. The statistics of our study, however, precluded an analysis of the angular dependence of radiation near to the Bragg peak. We therefore integrated the intensity in a reciprocal space volume  $V$  around the reflections, after correcting for the slowly varying background. This intensity is related

to the mean square displacement,  $\langle x_{sc}^2 \rangle$ , according to

$$I(\mathbf{k}) = D |F_0(hkl)|^2 e^{-k^2 \langle x_{sc}^2 \rangle} \quad (1)$$

where  $\mathbf{k}$  is the scattering vector of length  $4\pi \sin \theta / \lambda$  and  $F_0(hkl)$  is the structure factor of the ideal average molecule of the crystal. Only short-range-correlated motions within the unit cell are contained in  $\langle x_{sc}^2 \rangle$ . Provided there are no intermolecular motions correlated over distances greater than the linear dimensions of the unit cell, equation (1) gives the intensity of the Bragg peak, for which  $D = 1$  and 0 for the elastic ( $I_{el}$ ) and inelastic ( $I_{inel}$ ) components respectively. The presence of rigid-body molecular motions results in peaked diffuse inelastic scattering centred on the Bragg reflections. As these motions are correlated over a long range, their mean squared displacement shall be denoted  $\langle x_{lc}^2 \rangle$ . With  $D = (1 - \exp(-k^2 \langle x_{lc}^2 \rangle))$ , equation (1) then gives  $I_{inel}(\mathbf{k})$ , whereas the elastic intensity,  $I_{el}(\mathbf{k})$ , is obtained with  $D = \exp(-k^2 \langle x_{lc}^2 \rangle)$ . Thus, the ratio

$$f^R(\mathbf{k}) = \frac{I_{el}(\mathbf{k})}{I_{tot}(\mathbf{k})} = e^{-k^2 \langle x_{lc}^2 \rangle} \quad (2)$$

where  $I_{tot}(\mathbf{k})$  is the total intensity of scattered radiation entering  $V$  gives the mean squared displacement  $\langle x_{lc}^2 \rangle$ . For myoglobin, peaked inelastic intensity was found only near the reciprocal lattice nodes, from which a mean squared displacement  $\langle x_{lc}^2 \rangle$  of  $0.018 \pm 0.009$  Å was determined.

By contrast with Rayleigh scattering of Mössbauer resonance (RSMR), Mössbauer absorption spectroscopy provides a mean squared displacement,  $\langle x^2 \rangle$ , which includes both the short-range- and the long-range-correlated motions that are coupled to the iron atoms in myoglobin crystals. Figure 3 shows the temperature dependence of these  $\langle x^2 \rangle$  values. An extrapolation to room temperature of the linear behaviour observed at low temperatures agrees with the  $\langle x_{lc}^2 \rangle$  values obtained by RSMR. Although the error bars are rather large, this suggests that below 200 K rigid-body molecular motions make a significant contribution to  $\langle x^2 \rangle$ . Above 200 K, additional protein-specific modes of motion which are overdamped and short-range-coupled become thermally activated.

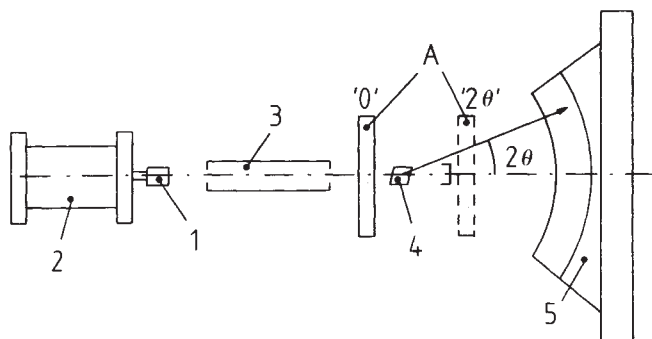


FIG. 1 The experimental arrangement. The Mössbauer source (1) (400 mCi <sup>57</sup>CoO) mounted on an electromagnetic driving system (2) is moved with velocity  $v$ . The collimator (3) confines the emitted radiation to a primary beam divergence of less than  $0.7^\circ$ . The scattered radiation from the myoglobin crystal (4) is collected by an area sensitive multiwire proportional counter<sup>10</sup> (5). To perform an energy analysis, a Mössbauer absorber (A) (a mixture of  $\text{Li}_3^{57}\text{FeF}_6$  and  $(\text{NH}_4)_3^{57}\text{FeF}_6$ ) is placed either in the incident beam between the source and the crystal (position '0') or into the scattered beam between the crystal and the detector (position '2θ').

**METHODS.** Count rates  $Z_p(v)$  were recorded at an angle  $2\theta$  under four different conditions: the resonant analyser was either in position  $p = '0'$  or  $p = '2\theta'$ ; the source velocity was either  $v_r$  or  $v_\infty$ . At  $v_\infty$ , resonance absorption was negligible and  $\gamma$ -rays passed through the absorber; at  $v_r$ , the absorber removed essentially elastic  $\gamma$ -rays. The total intensity of scattered photons,  $I_{tot}$ , was measured by  $I_{tot} = Z_0(v_\infty) - Z_0(v_r)$ , whereas the intensity of elastically scattered photons,  $I_{el}$ , was measured by  $I_{el} = Z_{2\theta}(v_\infty) - Z_{2\theta}(v_r)$ . The difference,  $I_{tot} - I_{el}$ , was proportional to the intensity of inelastically scattered photons.

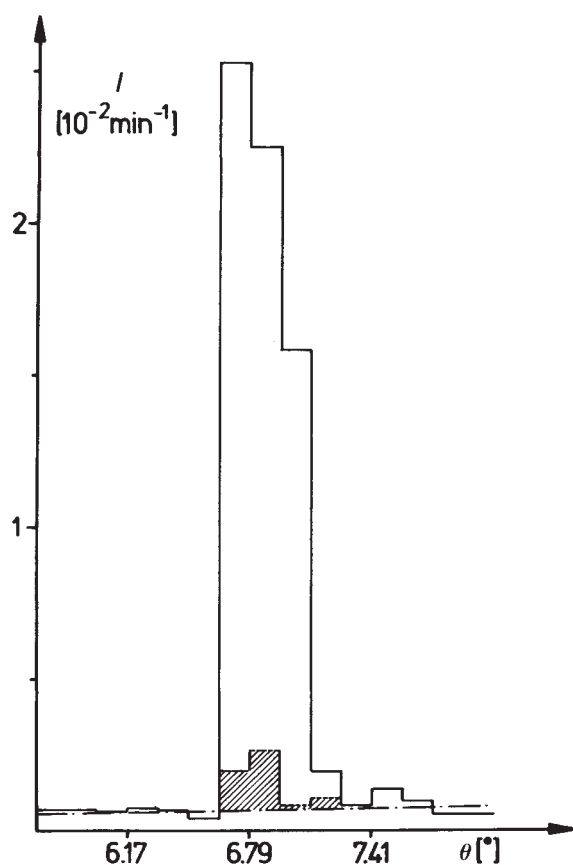


FIG. 2 Bragg reflection 16 2 1 from the myoglobin crystal: solid line, total intensity; hatched area, inelastically scattered intensity at the Bragg position; dashed-dotted line, correction for Compton effect and smoothly varying diffuse scattering.

The crystallographic  $B$ -factor also provides a mean squared displacement,  $\langle x^2 \rangle^x$ , which corresponds to the average deviation of all atoms from their ideal lattice positions. For myoglobin at room temperature a displacement  $\langle x^2 \rangle^x$  of  $0.158 \text{ \AA}^2$  has been determined<sup>8</sup>. This value does not include intermolecular motions because the inelastic intensity arising from these modes appears close to the reciprocal lattice node and cannot be separated in the evaluation procedure. The comparison with  $\langle x^2 \rangle^x$  again shows that motions coupled over short ranges within the molecules are responsible for the main part of the disorder. To determine whether these short-range-coupled displacements are due to static distortions of the molecules or to molecular dynamics, RSMR measurements on a sample containing a large number of small myoglobin single crystals have been made<sup>9</sup>. In this study elastic and inelastic intensities were integrated over spherical shells in reciprocal space, allowing the smoothly varying inelastic component of the thermal diffuse scattering to be included. Although the peaked inelastic component was only 5% of the total integrated intensity of the 16 2 1 reflection (without background), the inelastic scattering at the corresponding resolution of  $3.6 \text{ \AA}$  accounted for 56% of the total intensity in this shell. This large proportion can be explained only if the protein molecules perform intramolecular motions with large amplitudes in accordance with the X-ray results.

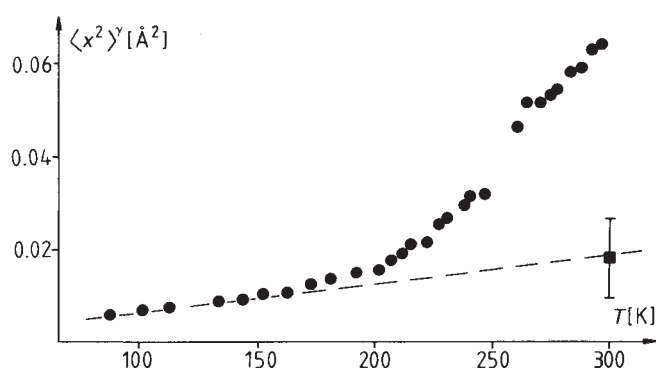


FIG. 3 Mean squared displacements of myoglobin: circles,  $\langle x^2 \rangle^y$  from Mössbauer absorption experiments<sup>11</sup>, square,  $\langle x^2 \rangle^x$  from the present experiment; dashed line, least squares fit to the low-temperature points. The extrapolation to room temperature gives  $\langle x^2 \rangle^y = 0.018 \text{ \AA}^2$ .

Our experiments constitute the first application of 'Mössbauer crystallography' to proteins. In the future it should be possible to obtain more information by simultaneously exciting many reflections with a divergent beam and collecting the data with an area-sensitive detector. A comparison of diffusely scattered Mössbauer radiation with X-ray scattering should provide insight into the molecular dynamics of many proteins in the crystal state. □

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## ERRATUM

### Identification of globular mechanochemical heads of kinesin

Jonathan M. Scholey, John Heuser, Joy T. Yang & Lawrence S. B. Goldstein

*Nature* **338**, 355–357 (1989).

AN error in Fig. 1 legend of this letter, in which MgATP was substituted for MgADP, caused the legend to be misleading. A correct explanation is: Kinesin from the Biogel column (lanes 1', 2') was incubated with apyrase to hydrolyse contaminating MgATP or MgADP (lanes 3', 4'). Identical results to those in lanes 1' and 2' were obtained in parallel digestion reactions performed in the presence of additional MgADP (1 mM) which yielded identical results to those shown in lanes 5' and 6'.



# Talents out of balance

P.E. Bryant

**Extraordinary People: Understanding "Idiot Savants".** By Darold A. Treffert. Harper & Row, New York/Bantam, London: 1989. Pp. 276. \$17.95, £12.95\*.

WE ARE used to a certain unevenness in people's intellectual attainments. None of us would be particularly surprised to meet a successful but innumerate novelist or a brilliant chemist who writes the most turgid prose. Sometimes, however, the imbalance between ability and inability within an individual is so pronounced that we have to take notice. Idiots savants, or 'savants' as they are called nowadays, demand that kind of attention. These are mentally retarded or autistic people with profound intellectual handicaps who manage nonetheless to acquire remarkable specific skills. Some become excellent artists; others musicians. Some make amazingly rapid mathematical calculations; others can work out in a matter of seconds the day of the week when a particular event occurred.

Darold Treffert has written the first comprehensive account of these astonishing people. His book starts with detailed histories of some of the most striking cases. These are written with gusto and they all inspire awe. He tells us, for example, of two profoundly handicapped men, with extraordinary musical memory. They can remember tunes that they have only heard briefly and can reproduce them on the piano with consummate ease. Yet one is blind, paraplegic and has an IQ of 58, and the other, with an IQ of 62, is severely autistic. There are descriptions too of autistic children who become skilled artists at an early age. One of them is an autistic girl, Nadia, who at the age of six was able to draw pictures of animals and people in movement that are so realistic and powerful that it is almost impossible to believe that they had been done by someone so young: and yet Nadia at the time was so autistic that she could not even speak. The obscure gifts of the calendrical calculators who can work out the day of the week when an event took place are also set out here, although we are told very little about the rules that they use to make these calculations.

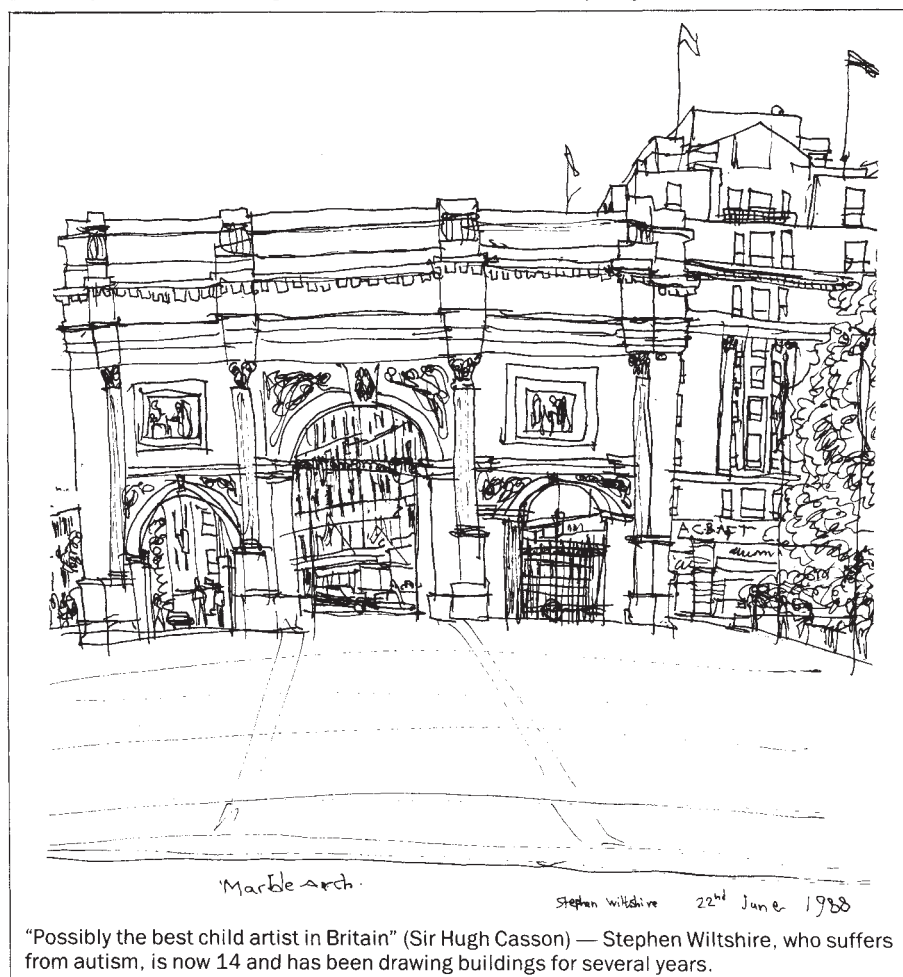
The bulk of the book is taken up with detailed, well-written case histories. Treffert's lively interest in savants is infectious, and no one could read what he has to say about the individuals concerned without wanting to know more about the reasons for this condition. Treffert recognizes that the existence of savants poses important theoretical questions, which he tries to answer in the last part of the book.

He suggests that savants develop their remarkable gifts because they have an unusually good memory. There may be something in this: individual savants obviously do have prodigious memories for visual scenes (if their talent is for drawing) or for tunes (if they are musicians). But these extraordinary feats of memory are linked to the specific skill which the savant happens to have. Treffert dwells on the fact there is some evidence that savants have a better memory in general than other people of the same intellectual level, but the truth is that the absolute level of the savants' scores in general tests of memory is rather low and could not possibly explain their prodigious talents. There are even limitations to their memory for material associated with their skill. One of the savant musicians (studied by Sloboda, Hermelin and O'Connor) remembered a piece by Grieg which he had just heard for the first time almost perfectly. But the same person did rela-

tively poorly when he had to remember and reproduce an unusual and slightly atonal piece by Bartok.

Treffert also has a neurophysiological explanation for the condition. He claims that it is usually due to damage to the left hemisphere and consequent compensation in the development of the right hemisphere of the cerebral cortex. However the evidence that he offers for this hypothesis is thin. Scans done with computed axial tomography have shown that some savants have indeed suffered damage to their left hemisphere, but the idea of compensation in the right hemisphere remains pure speculation.

No one has yet produced a convincing explanation for the savants' talents, and so one should not chide Treffert for not managing to do so either. But he can be criticized for not spending enough time discussing what the problem really is. One must start with a simple question. We are not surprised by mild imbalances in the pattern of a person's skills (the novelist who cannot add well) and yet we are amazed at more striking imbalances (the concert pianist who is blind and retarded). Why is this so? The answer is that we expect a positive, but not a perfect, correlation between different intellectual skills. Mild imbalances do not violate this expectation: very large imbalances do.



From Extraordinary People

\*In Britain the book is subtitled *An Exploration of the Savant Syndrome*.

There are two possible approaches to the extreme intellectual imbalances that we find in savants. One is to argue that the idea of the positive correlation is wrong, at any rate as far as musical and artistic skills, and the ability to make arithmetical calculations, are concerned. These skills are independent: they are not affected one way or the other by our other intellectual abilities. The second approach is to accept the positive correlation as a general proposition but to argue that it breaks down in savants. They are a special case because the special nature of their disability leads them to concentrate on a particular skill at the expense of virtually everything else.

There is a pronounced difference between these two approaches. According to

the first, the existence of savants tells us about the organization of intellectual skills in general. According to the second, we can only learn from them what kind of compensation is possible after early damage to the central nervous system. Treffert never makes a clear distinction between these two possibilities. That seems to me to be the reason why his book, which starts off so well with the description of these remarkable people, ends disappointingly with a failure to establish what savants tell us about the workings of their and our intellects. □

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## How to win a Nobel prize

Steve Blinkhorn

**Scientific Genius: A Psychology of Science.** By Dean Keith Simonton. Cambridge University Press: 1989. Pp.229. £22.50, \$27.95.

Now calm down ladies and gentlemen. There is about as much connection between *Scientific Genius* and *Teach Yourself Nobel Prizewinning* as there is between a textbook on stereochemistry and *The Joy of Sex*. Here we have not so much an account of how to do it, as of what makes other people do it. And even then the answer appears to be that Other People Do It At Random.

Well, perhaps not quite. If you really want to make your mark today as a scientific genius, it helps to be a firstborn, displaced Jewish orphan brought up in a middle-class cultured household in the United States, and to have a moderately high IQ. But then your influence over these factors is more or less restricted to the possibility of murdering a parent or two, and even that won't help much at your age.

More to the point, Simonton sets out to dismantle heroic and romantic theories of genius and replace them with a theory of his own, the 'chance-configuration' theory. So the book is structured as a statement of the theory followed by an examination of the extent to which such evidence as can be adduced supports it as compared with the alternatives. No one — the author included — would claim that these comparisons are based on a rigorous methodology or on watertight data sets. But plenty of ideas are sketched out, and interesting (if tendentious) quantifications suggested. For example it seems that creative potential is related to age by the formula  $x = 305e^{-0.004x}$ . Also

included is a good, comprehensive bibliography which perhaps predictably contains 54 of Simonton's own publications.

Indeed, quite a lot of the value in the book is in its survey of the many, various and often almost mystical ideas that have been pressed into service to explain the phenomenon of genius. Although thorough, it is not a deep examination, and much is asked of the reader in terms either of previous knowledge in the field or of trust in the author's elliptical references to the literature. The book shows every sign of being precisely what it is, the product of a specialist's sabbatical freedom (and none the worse for that unless you are looking for a *good read*).

So what of the 'chance-configuration' theory of genius? I found myself suddenly coming over to the author's side on page 198 with the statement that "much of the current psychology of science has misplaced its emphasis on rational cognitive heuristics". Socrates had it wrong: man is merely an animal capable of occasional bouts of rationality, and maybe his rational moments are not his most creative. What Simonton is saying is that a theory of genius has the same general form as a theory of constellations or a theory of faces in the fire — which is to say no real theory at all. The more elements you have, the more complex the patterns you can see. And constellations have been known to guide space-ships. But whatever the processes of scientific creativity, genius is recognized after the event, and is an attribution of social recognition not a quality of thought.

All of which is a little sad for those who would like a do-it-yourself eminent-achievement-by-numbers kit. Because one is drawn to the conclusion, when all is said and done, that genius, like happiness, is destroyed in the pursuit thereof. □

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## Psychology's Johnson

J.D. Mollon

**Macmillan Dictionary of Psychology.** By Stuart Sutherland. Macmillan, London/Crossroad-Continuum, 370 Lexington Avenue, New York, New York 10017: 1989. Pp.491. £29.95, \$49.50.

Among these unhappy mortals is the writer of dictionaries; whom mankind have considered, not as the pupil, but the slave of Science, the pioneer of literature, doomed only to remove rubbish and clear obstacles from the paths of Learning and Genius, who press forward to conquest and glory, without bestowing a smile on the humble drudge that facilitates their progress. Every other author may aspire to praise; the lexicographer can only hope to escape reproach, and even this negative recompense has yet been granted to a very few. [Samuel Johnson, *A Dictionary of the English Language*, 1755.]

PSYCHOLOGY has attracted its share of dictionary makers, quite a drove of them in the past two decades. But some have wanted industry, others understanding; and none of their compilations has been truly satisfactory. In Stuart Sutherland the discipline has now secured a worthy lexicographer. And there are not a few parallels between Johnson and Sutherland: they enjoy the same robust good sense; they share a somewhat choleric style; and both are men who have placed their private melancholia in the public domain.

Dr Johnson was blunt in deflecting criticism of his dictionary: "Ignorance madam, sheer ignorance" was his response when asked why he had defined *pastern* as the knee of a horse. And Sutherland follows, writing in his preface: "It is customary for dictionary writers to acknowledge that their work is likely to contain mistakes, and to ask readers to write pointing out any they encounter. I apologise for any errors that have crept into mine, but I beg the reader not to draw my attention to them . . .".

I will here respect Professor Sutherland's sensibility, but if the sales of this excellent dictionary prompt an early reprint, then I shall be pleased (for a professional fee) to supply to the publisher a list of more than 20 errors of substance. For the present, I must needs confine myself to Preterition and shall not take our Lexicographer to task for confounding Ideal and Standard observers, for blurring the hard-won distinction between Intervening variables and Hypothetical constructs, for failing to differentiate Short-term memory and Short-term store, or for neglecting the asymmetry of the Stroop effect. I shall even pass over the misleading entry for Forced choice, an entry that fails completely to acknowledge Tanner and Swets'



classical distinction between Yes-No and forced-choice experiments.

In fact, the number of flaws in the dictionary is tiny. Sutherland's especial talent lies in using plain language to give a succinct definition of complex concepts. His economy of words is often marvellous. And when usage is vague or when a term is empty of meaning, he does not hesitate to tell us.

One of the criteria for judging a dictionary has to be the comprehensiveness of its coverage. Sutherland's coverage is very good, though not perfect. He explicitly intended his book to be a dictionary for psychologists, in that he includes many terms from related disciplines. Statistics, neuroanatomy, linguistics,

classical genetics, psychoanalysis and optometry are notably well covered; and he is fairly comprehensive on the more curious sexual practices. But these extensions may be at the expense of the core of our discipline. Thus Ovarian follicle and many other gynaecological terms are included, but the psychological reader will look in vain for AB error, Additive factors method, Bidwell effect, Cohort model, Liebmann effect, Memory-scanning task, Molyneux's question, Ranschburg phenomenon, Repetition effect, Transitional probability and Wason task. And even within a category there are unevennesses. Thus Hampton Court maze is in, but Olton maze is not. The antique Holmgren test is in, but the Geller-Seifter test is missing. Tribadism, Frottage and no less than four variants of Cunnilinctio are in, but some old faithfuls, such as Cunniphagia, Ligotage and Irrumation are taboo.

Sutherland enlivens his dictionary with two jokes (although he uses them needlessly often). They are the two jokes used by Johnson, viz:

"social facilitation. The facilitation of behaviour by conspecifics . . . does not apply to certain complex tasks, like compiling dictionaries" (Sutherland). Compare: "dull . . . Not exhilarating; not delightful; as, to make dictionaries is dull work" (Johnson).

"psychoanalyst. A person who takes



The dictionary-maker depressed. Perhaps today he would be recognized as suffering from Aerophagia: "Swallowing air, a common neurotic habit that can produce discomfort and belching".

money from another on the pretence that it is for the other's good" (Sutherland). Compare: "patron . . . Commonly a wretch who supports with insolence, and is paid with flattery" (Johnson).

Sutherland uses the second of these jokes (*mutatis mutandis*) to convey his jaundiced view of cognitive scientists, social scientists, Gibsonians, Skinnerians and the sillier kinds of psychotherapist. And in general, it is a depressing view of psychological science that emerges from his dictionary. What becomes manifest is the lack of system, the categorical anarchy, with which we today approach the study of the mind. Psychologists have little to call their own except a ragbag of experimental paradigms and a heterogeneous collection of vague explanatory terms such as 'arousal' and 'drive'. For the rest, we depend on borrowings from other disciplines.

There is no better way of commending this book than to quote again from the choleric Doctor: "The words of this dictionary, as opposed to others, are more diligently collected, more accurately spelled, more faithfully explained, and more authentically ascertained" (*A Dictionary of the English Language*, preface to the eighth edition).

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## Fighting talk

Roy Porter

**AIDS and Its Metaphors.** By Susan Sontag. Farrar, Straus & Giroux, New York/Allen Lane, The Penguin Press, London: 1989. Pp.95. \$14.95, £9.95.

DISEASE kills, but fear of disease can be almost as deadly. So argues the distinguished American intellectual, Susan Sontag. Ten years ago, in her *Illness as Metaphor*, she laid bare our society's dangerous habit of spinning fantasies around certain diseases (leprosy, plague, tuberculosis, and so on), thereby creating terror and guilt. In particular she denounced the folklore of cancer, the popular image of 'the big C' as untreatable, invariably fatal and, above all, psychogenic — the product of the so-called 'cancer personality', the self that eats itself away through frustration and repressed anger. Such myths made cancer unmentionable and created terrible stigma: through them therapy was hindered and suffering multiplied. We must abandon the phony meanings we attribute to sickness and the metaphors that sustain them, insisted Ms Sontag, and look disease squarely in the face.

*Illness as Metaphor* was a brave book (particularly as Ms Sontag was herself suffering from cancer), and it performed valuable service in combating prejudice. Her new book, entitled *AIDS and Its Metaphors*, must be read as a kind of extended epilogue to that work. She is still a campaigner against dangerous nonsense, but now her target is the mythology growing up around AIDS: new, deadly and still without effective therapies, AIDS is precisely the kind of disease that spawns pseudo-explanations. Popular moralists and the media have had a field day in labelling it nature's punishment for promiscuity, or God's revenge against gays and drug addicts. Phony aetiologies are invented which reinforce wider demonologies. It must all have started, rumour has it, as a CIA plot, or as one of the KGB's dirty tricks; or it is just another nasty thing coming out of the 'dark continent'.

Slipshod thinking such as this creates new cohorts of pariahs. Thanks to the tricks of language, being HIV-positive easily becomes the same thing as 'having AIDS', with all too serious consequences for people's jobs and lives. And, not least, the metaphor of disease as a deadly foe generates a miasma of panic. If infection is seen as the ultimate, insidious 'enemy', no door knob, no toilet seat, is safe. Because cold war and 'Star Wars' propaganda makes popular paranoia so pervasive, it is all too easy to treat AIDS sufferers as the enemy within. We must disabuse ourselves of such language-fuelled phobia. ▶



Ms Sontag's sentiments are admirable and are eloquently expressed. But her points have already been made hundreds of times over the past few years — the whole book evokes a wearying sense of *déjà vu*. And Ms Sontag fails to get off her intellectual high horse, and descend to nitty-gritty practical problems. If metaphor is duplicitous, if we must stop using 'military metaphors' such as 'fighting disease', how on Earth are medical educators to get their message across to the public? If (as she rightly emphasizes) fear and prejudice stigmatize sufferers, how are we to instil that salutary fear of infection which is necessary to encourage

safer lifestyles? Surely there must be good metaphors as well as bad, true ones as well as false. After all, we have to use language, and what speech is not (as one might say) 'infected' with metaphors?

Like so many intellectuals, Ms Sontag seems so lost in the web of words that she is in danger of having nothing useful to say about (what she finds an offensive metaphor) conquering disease in the real world. □

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## Burden of proof

Beverly E. Griffin

**AIDS: The HIV Myth.** By Jad Adams. Macmillan, London/St Martin's Press, New York: 1989. Pp.244. £12.95, \$16.95.

"PESTILENCES have a way of recurring in the world; yet somehow we find it hard to believe in ones that crash down on our heads from the blue sky" (Albert Camus, *The Plague*). Camus was describing a fictional plague that originated from a dormant organism which arose unbidden in an Algerian town in the 1940s. Later, in an intriguing piece of prognostic fiction from the 1970s, an author called Russell Foreman produced *The Ringway Virus* which threatened the human race. (It was traced to a single outbreak in Australia and differed from all known viruses in its speed of spread and deadliness of action.) In the 1980s, the virus of the moment is undoubtedly HIV. The epidemic is AIDS. But is this a matter of cause and effect?

Jad Adams, a journalist with a claim to scientific respectability, has written a meticulously researched book whose title leaves little doubt about his answer to the question. The implication of the title is reinforced by inclusion of a foreword by Peter Duesberg, the most articulate of those who are sceptical that HIV is the cause of AIDS. Adams summarizes his conclusions thus: "my view, informed only by literature on the subject . . . is that HIV does not cause AIDS. Another pathogen . . . is the causative agent. . . . We will find out whether HIV causes AIDS but I predict this will happen a long way into the future and few will emerge from the historical record with much distinction".

Here I have jumped from the title page to the end of the book. What lies in between are a number of highly readable chapters on the origin of AIDS and the syndrome itself, the four Hs (haemophiliacs, heroin users, homosexuals,

Haitians) who have proved most susceptible to the causative agent (whatever it may be) and the fifth H (hookers) who might by now have been expected to join the statistics on AIDS, but have not. To my mind, the most thought-provoking chapter is that simply entitled "Syphilis". Here Adams lays the groundwork for his contention that an organism like the spirochaete *Treponema pallidum* should be seriously considered as a contributing factor to AIDS: symptoms of AIDS parallel those of syphilis; many patients are said to respond to antibiotic therapy (conventional viruses do not); and syphilis in some stages is immunosuppressive. "The great masquerader" might even exist in a filterable form, filtration being one of the prime pieces of evidence, in lieu of any animal model, for assuming AIDS is caused by a virus. If Adams wants support for his notions about syphilis he may find some in statements taken from the World Health Organization (see *The Lancet*, 18 February 1989, p.396) on sexually transmitted diseases as risk factors for HIV transmission.

What about HIV? Adams's response to the "myth" is a chapter called "HIV Challenged". He also presents the whole sorry story about the politics of its discovery, the people involved, the mistakes that occurred, the failure of Richard Nixon's war on cancer and so on. Scientists in general do not fare too well at Adams's hands, nor do grant-giving bodies. He quotes from Paul Feyerabend: "as opposed to its immediate predecessor, later twentieth-century science has given up all philosophical pretensions and has become a powerful business that shapes the mentality of its practitioners". Named scientists are the butt of Adams's invective as he draws attention to their abandonment of the scientific approach, acquisition of vested interests in the virus they champion and use of steam-roller tactics to silence any opposition. In "Virus Hunters" he reveals a Gallic bias. The AZT story, a medical trial that was neither anonymous nor completed but resulted in the release of a drug for AIDS treatment,

is also discussed in a further chapter entitled "Finance".

Many will say that this book is totally flawed. I will say it has minor flaws. Adams concentrates too forcefully on the extremes — HIV *is* or *is not* the cause of AIDS — and too little on the middle position wherein HIV is seen as a contributing factor to the disease(s) with other, as yet unidentified, cofactors also being required. (This is not fence-sitting — there is a precedent for the involvement of a latent virus, the herpesvirus EBV in this case, together with cofactors in several human malignancies.) One could even hope that HIV *is* involved because, in the normal sense, it is a relatively non-infectious virus. Therefore changes in life-style and the use of safer blood products (for the five Hs and the potentially large 'at risk' African population) could result in a decline in the AIDS epidemic.

Adams also falls into the trap (following Duesberg) of assuming that the presence of HIV antibodies should in fact mean vaccination protection for the patient. Again, to quote from the EBV literature, increased viral antibody titres are used diagnostically to predict people at risk of developing EBV-associated diseases. Thus, not all viral antibodies can be deemed to be markers of protection. Finally, as one who works with young scientists and medical technicians, I regret, and in part strongly disagree with, Adams's cynical assessment of the 'scientific method'.

These, however, are minor points. Adams has written a highly provocative but important book on a huge medical problem. It deserves to be read (even by Nobel committees). There must be many 'HIV=AIDS agnostics' who will feel a great sense of relief if this book frees them from the pressures of silence imposed by the establishment (including journalists and journals). It will surely lead to a scientifically healthier society if the burden of proof for HIV as a deadly pathogen is returned to where it belongs — to those who maintain that HIV causes AIDS — and others are allowed to pursue alternative approaches in the battle for eradication of the disease. □

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• *AIDS: Profile of an Epidemic*, recently published, is more specific in scope than the title implies, in that the contributors deal largely with AIDS in the Americas. The book has, however, been published with commendable speed; it contains a great deal of up-to-date information, much of it pertaining to South and Central America. Publisher is the Pan American Health Organization, whose publication centre is at 49 Sheridan Avenue, Albany, New York 12210. Price is \$30, plus shipping and handling. A Spanish edition is also available.

# Tootsie afoot?

Stephen Lock

**The Health Conspiracy: How Doctors, the Drug Industry and the Government Undermine Our Health.** By Joe Collier. *Century Hutchinson, London: 1989. Pp.175. Pbk £4.95.*

"PRACTICALLY none of the great therapeutic advances has come from practising doctors or departments of clinical research" — that contention is as true today as when it was first made 20 years ago by Lord Platt, a professor of medicine and president of the Royal College of Physicians. Yet fortunately, since the therapeutic revolution began with the sulphonamides in the 1930s, the pharmaceutical industry has developed a massive armament of drugs against common disease, including not only the antibiotics but treatments for peptic ulcers, raised blood pressure, heart disease, asthma, diabetes, Parkinson's disease and schizophrenia.

At the same time all of us have been uneasily aware of the negative aspect of this success story — the inescapable side-effects of many drugs (some horrendous) and the sleazy way in which some drugs have been promoted. In Britain, and elsewhere, has this difficult balance now tilted the wrong way? It is Joe Collier's contention that it has: not only is the pharmaceutical industry becoming less innovative as research is increasingly diverted to commercial ends, but there is a conspiracy by "rich and privileged forces" who are denying patients the treatment they need.

## Prejudice

To be sure, Collier, an academic clinical pharmacologist who works in London, phrases the last claim as a question. But he leaves us in little doubt about his own answer. As part of the rich and privileged forces, British doctors are failing their patients. Not only are their judgements contaminated by prejudices — those of class, sex and race — but they have lost their touch and can no longer make diagnoses with confidence. Rather than tackle the root causes of illness, or use supportive treatment, they keep patients at arm's length, reflexly reaching for the prescription pad. And they are in the pocket of the drug industry, now the dominant influence on medical practice. Casting its net wide, not only does the industry spend £140 million a year on drug promotion — including gifts to doctors — but some of its so-called research expenditure includes bogus drug trials — post-marketing surveillance designed so that family doctors prescribe the drug under study — and seminars that are barely concealed public relations exercises.

All this is stark stuff, though Collier is

careful to document most of his allegations. Yet there is a third conspirator in his indictment: the British government. At the heart of *The Health Conspiracy* is the CSM, the Committee on Safety of Medicines, which arose out of the public anguish over the thalidomide affair. Today, obtaining a licence for a new drug may take up to 12 years of a 20-year patent life, and the members of the CSM, who take the decision, may have to assess (literally) a lorry load of data. Yet they are concerned with safety and not efficacy (which is the remit of another committee), and moreover with balancing the risks of a drug against the dangers of the disease. It is their task to monitor the safety of a drug after it has been licensed, but that process is inevitably imperfect and tragic instances of unsuspected lethal side-effects still make the headlines.

Collier concedes that, worldwide, the CSM has been one of the most successful regulatory agencies but argues that it is hamstrung in three major ways. First, its composition, with five members from the drug industry and several medical academics with paid consultancies, raises questions of conflicts of interest. Second, its restricted concern with safety means that it is flooded with applications to license look-alike drugs which have no advantages over those already on the market. Third, given that it is part of the Department of Health's Medicines Division, not only is it caught up in the national obsession for secrecy about almost everything, but there is also a "hopelessly conflicting" contradiction in the department's dual role of regulating drugs, on the one hand, and promoting a successful international drug industry on the other.

An eminently readable book, *The Health Conspiracy* deals well with its subject. There is a succinct account of the complexities of drug licensing as well as side-swipes about smoking, alcohol, screening for cervical and breast cancer, and the Black Report of 1980, *Inequalities in Health*. Collier also offers solutions to the main problems. Principally, a new alliance between patients and providers is needed. Scientists in the drug industry must remember that their first duty is to science, while marketing and promotion staff must ensure that advertisements are honest. The government should divorce the two functions of the Medicines Division, transferring the promotional side to the Department of Trade; revise the Medicines Act to ensure openness and an emphasis on efficacy; and establish a list of patients' rights, including no-fault compensation. Doctors must become more vigilant in dealing with the industry and reform their training. Patients must take a more active role in their own treatment by asserting their rights.

Although a recent White Paper on the future of the National Health Service has

made a few of Collier's facts out of date, most of them are right; it is his interpretation of them that I find more difficult (and here I should declare an interest in editing a journal that is partially funded by pharmaceutical advertisements).

## Idealism

Collier is evidently an idealist. Part of his dissatisfaction, it seems to me, stems from disenchantment with human nature, part from a dislike of specific features of British life. No society has found a better mechanism than capitalism for developing new ideas — how many valuable drugs have come out of the Eastern bloc countries? — and capitalism entails an inevitable tension between purity and commerce. In Britain, moreover, there has long been a syndrome of *dolce far niente*, which has been tolerated by successive generations and governments of all complexions. As well as those described by Collier, its features include variously the low fraction of gross national product devoted to the National Health Service, the low proportion of school leavers receiving higher education, the Official Secrets Act, overcrowding in prisons, and the tower blocks on the Aylesbury estate. To these we can add the tootsie-footsie relationship between doctors and the pharmaceutical industry.

All this is not to say that several aspects of the pharmaceutical industry are not distasteful, or even dishonest, and eventually most of them will have to be put right (as has happened in the Nordic countries). But in life you are either liberal with Locke or coercive with Hobbes, and my personal preference is for the former. Part of my fears of excessive reform stem from the certainty of those who recommend it, and the possibility that individual clinical decisions will come to be made by diktat.

Thus I am dismayed by one feature of a publication that Collier commends — the *Drug and Therapeutics Bulletin* — and that is censorship. Sent by the Department of Health free to every general practitioner in England and Wales, the *Bulletin* is a valuable summary of the uses, side effects and contraindications of drugs old and new. It is written by anonymous experts and extensively reviewed before publication. Yet it has no mechanism for debate; it publishes no correspondence and only the occasional correction of fact; all this on subjects where there can be no certainty without the widest discussion. Open debate is, I believe, still the one big plus in our society. We should hesitate before destroying the creativity of the pharmaceutical industry. And that, I suspect, would be the result if Collier's suggestions were implemented. □

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# Protean evolution

Jerry A. Coyne

**Macroevolutionary Dynamics: Species, Niches, & Adaptive Peaks.** By Niles Eldredge. McGraw-Hill: 1989. Pp.226. Hbk \$28.95; pbk \$14.95.

EVOLUTIONARY biologists have found it hard to wrestle with the theory of punctuated equilibrium, for, like Proteus, it changes form when firmly grasped. Having gradually abandoned many of the key concepts, its proponents still insist that it is a revolutionary explanation of macroevolution, the large-scale changes in the types and numbers of organisms during the history of life.

Punctuated equilibrium's newest incarnation, described in *Macroevolutionary Dynamics*, is missing further parts. Gone are the ideas that evolutionary stasis is caused by ontogenetic resistance to change, that key evolutionary innovations begin as maladaptive features fixed by genetic drift, that macromutations play a large role in evolution, and that evolutionary trends result from species selection. Although the new, truncated theory is barely recognizable as punctuated equilibrium, Eldredge implies that it has not changed much since the controversial original version of 1972. But those who have followed this controversy will be astonished at Eldredge's revisionism, including the assertion that he and Stephen Gould always "remained neo-Darwinian gradualists in the sense that the brief periods of relatively rapid change involved intergradational, rather than saltational, phenotypic transformation, presumably under the control of natural selection" (p.66).

This short volume, apparently written for those with a professional interest in evolution, describes the two remaining tenets of punctuated equilibrium. The first is the familiar idea that species change very little over most of their evolutionary history. This stasis is no longer attributed to developmental homeostasis, but to a combination of behavioural habitat selection (organisms seek out familiar niches when the environment changes, therefore experiencing no new selection) and disruptive natural selection (different populations adapt to different local environments, and the 'average' environment among populations of a species does not change).

These ideas may correctly explain unchanging species, but they certainly do not predict them. Not many plants, for example, can avoid unpleasant environments. Even in animals, habitat selection causes stasis only when a familiar habitat can be found in a changing world, but this must often be impossible (why else is there extinction?). Disruptive selection is even less plausible, for the argument assumes

that there is never any change in the 'average' habitat among populations, and that directional selection cannot apply over a species' entire range. The former idea seems unreasonable, and the latter clearly wrong. It is easy to think of geographically widespread forms of natural selection, including the introduction of new parasites or diseases, selection for crypsis, sexual selection and mutations that increase fecundity.

The second tenet — and the only non-darwinian one — is that large changes of morphology are allowed only by speciation. There are, of course, good darwinian reasons for expecting an *association* between morphological change and reproductive isolation. Natural and sexual selection can cause reproductive isolation as a by-product, the classical explanation of adaptive radiations. Also, as Douglas Futuyma has noted, when a population becomes reproductively isolated it may keep adaptations that would normally be diluted away by gene flow from the rest of the species. This would likewise cause an association between speciation and morphological change. But Eldredge proposes something more radical: that reproductive isolation actually *triggers* adaptive change in geographically isolated populations.

Reproductive isolation is, however, a property of pairs of species that is seen only when they become sympatric, and it is difficult to see how its evolution in allopatry could somehow cause a species to acquire new adaptations. Indeed, Eldredge himself is hard pressed to come up with an explanation, suggesting only that new species could evolve rapidly by competing with their relatives. But such character displacement will affect only adaptations that reduce competition, and fails to account for many other features, especially those distinguishing related but geographically isolated species (for example, flightlessness in island birds). Because Eldredge has largely abandoned the only other non-darwinian part of punctuated equilibrium (species selection), the weakness of this argument brings down the last barrier separating his theory from neo-darwinism.

Unless *Macroevolutionary Dynamics* was intended as yet another revision of punctuated equilibrium, the reasons for the book's existence are unclear. Most of the material has been widely discussed elsewhere, the only novel element being a long and rather abstract discussion of the role of communities and ecosystems in evolution. The extensive philosophizing, as well as the dearth of biological examples supporting Eldredge's arguments, suggest that punctuated equilibrium is gradually losing its connection with the real world of organisms. □

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# Mischance for Mr Darwin

A.J. Cain

**The Correspondence of Charles Darwin, Volume 4 1847–1850.** Edited by Frederick Burkhardt and Sydney Smith. Cambridge University Press: 1989. Pp. 711. £32.50, \$32.50.

THE fourth volume of Charles Darwin's correspondence covers a period of four years in which, although he was intensely active, comparatively little went on of obvious excitement for the reader. Very little of this volume will give material for sensationalist literary writers (although I can recommend Darwin's use of chloroform in childbirth and his heroic remedies for toothache — did they simply kill the tooth?). This was one of those periods of heavy slogging work, with slow progress, and doubts developing about earlier ideas, that make up so much of any working scientist's life, and are usually characterized afterwards (sometimes rightly) as periods of consolidation. He had already sketched out his ideas on evolution, and published his *Journal* and his geological volumes. In the present period he published his contribution to the *Admiralty Manual of Scientific Enquiry* (1849).

All the usual mischances of life were bothering him in the usual way. Parcels of priceless specimens on loan to him went astray, guests had to be instructed on how to get to Down, references were required for friends for various jobs (he refused flatly to write one for someone he didn't know), there were difficulties over shares and farm rents and Emma Darwin's trust fund, his artist was (he thought) inexcusably dilatory, the printers of the *Admiralty Manual* made a hash of his contribution. But in addition his health was often very poor, and much time was spent on treatments which made only temporary improvements. His wife's pregnancies were sometimes difficult, scarlet fever attacked the family, one child had a convulsive fit, and his deeply respected father died. No wonder that he reported to Hooker on one occasion that "all things [have] gone on badly".

All this time he was keeping up his vast correspondence — with nurserymen and amateur breeders about variation, hybridity and inheritance, with other naturalists on effects of acclimation, taxonomic variation, geographical distribution, coral reefs, transported boulders (erratic blocks), the maximum inclination at which a lava stream could solidify, craters of volcanoes, raised sea beaches, salt from salinas, rock cleavage and foliation, ripple-marks on the sea bed at various depths, and especially the so-

[Down]

 My dear M<sup>r</sup> Innes

I am extremely sorry to hear of your toothache.— You must not put, I think more than *one* drop of Chloroform on the tooth.—<sup>3</sup> I send Tincture of Arnica<sup>4</sup> which smarts the skin (*deadly Poison*) to put *outside*.— M<sup>rs</sup> Darwin finds hot fomentations do best.— Many find cold water applications best.—

I have found two or three drops of Alum & Sw<sup>t</sup> Spirits of Nitre<sup>5</sup> (in bottle with a label) *sometimes* do my teeth *great* good. I was not in when your note came

Yours | C. Darwin

I send my bottles which you can return afterwards

 I send *Creosote*,<sup>6</sup> some find a drop of this do much good

Kill or cure — Darwin's sympathetic note to John Innes, Vicar of Down in Kent where the Darwin family lived from 1842, written in January 1848. (Reproduced from *The Correspondence of Charles Darwin*, Vol. 4; footnotes not included.)

called roads of Glen Roy. These are parallel lines on the hillsides of Glen Roy, near Ben Nevis, Scotland, marking successive levels of a former lake dammed up by a glacier.

But his principal activity was his work on barnacles, which, however, was moving into a more humdrum phase. His great discoveries were already made, and it was now a matter of completing his species descriptions, settling his taxonomic ranks and (most wearisome of all) determining his specific and generic nomenclature. This led to an interesting exchange of letters with Hugh Strickland over the principle of priority in nomenclature. Darwin thought it would favour bad and hasty work. He was in fact right. But Strickland had the better grasp of the art of making rules.

Michael Ghiselin, I believe, was the first to point out the importance of Darwin's work on barnacles, in his *Triumph of the Darwinian Method* (University of California Press, 1969). Up until then it had usually been ignored as something of an aberration, largely (I suspect) because historians and philosophers were incapable of understanding it. The reader is ably assisted in the present volume by a 22-page essay, *Darwin's Study of the Cirripedia* (it is Appendix II), which oddly does not refer to Ghiselin and has no author's name. Both the essay and the correspondence support Ghiselin's thesis of the importance of the work in confirming and testing Darwin's ideas on evolution. In particular, the extraordinary intermediates between the hermaphrodite condition and that with separate sexes served to strengthen previous ideas of his on the corresponding evolutionary modification of hermaphrodite flowers.

One wishes desperately for similar aids to understanding the controversies over the roads of Glen Roy, erratic blocks and volcanic craters. Darwin's correspondence with Lyell and Milne over Glen Roy is necessarily highly allusive to what they knew or had published, and correspondingly highly cryptic to a modern reader

who has not been over the actual ground in the company of a Quaternary geologist. Clearly, it is impossible for the editors to explain all these topics as well as the barnacles — one can only be grateful that the essay on them was included. The volumes of correspondence are the essential basis for starting on such explanations, which must be a collective effort independent of this series.

Moreover, the volumes of correspondence will be invaluable for assembling a series of real-life case-histories of what can go wrong in research. Failures of

classification (Darwin ignored some of the roads because he thought they were only sheep tracks), correct argument but from too narrow premises (as over dykes and eruptions), facts inadequately ascertained (the actual heights of the roads), or just plain wrong (Goodsir's description of a parasite as the male of the barnacle), inadequate observation for lack of a directing hypothesis (some of Hooker's geological observations in the Himalayas) — almost every practical and theoretical mistake that can occur in research could be illustrated authentically from these topics. A *catalogue raisonnée* of them would form an admirable manual of admonition for young research workers.

This volume, like its companions, is well produced and edited, and includes massive supporting notes, a concordance of the letters, lists of manuscript annotations, and family trees. Appendix I is a chronology from Darwin's original notebook, recently discovered; Appendix III gives his original observations on his children; and Appendix IV is his original notes of works to be read and works read, now published complete and with its own bibliography (which it needs). An excellent piece of work. □

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## Range finder

Henry Gee

**Dinosaur Plots & Other Intrigues in Natural History.** By Leonard Krishtalka. *Morrow, New York: 1989. Pp.316. \$17.95.*

THE West, apparently, "is where men are men and the plumbing is outside". It is also full of field palaeontologists like Leonard Krishtalka, who, when not actually digging things up in Wyoming (where the bar in the Big Horn Hotel in Arminto "seats four and leans five"), writes for the "Missing Links" column in the Carnegie Institute's bimonthly *Carnegie Magazine*. Twenty-five of his more off-beat contributions have mosied their way into this book, but Krishtalka's editors have made them wipe their boots first.

This is an irreverent collection of essays, far from the scholarly minutiae of Stephen Jay Gould's "Natural History" column or Carl Sagan's cosmic epics. Isaac Asimov could have written them if he'd been raised in a tin shack in Idaho rather than a sweetshop in Brooklyn — popular science, yet spiced with the kind of sleeves-rolled-up aphorisms one might expect in the writings of a bone-hunting man.

We learn, for example, that the only

spirits worth investigating come in bottles marked 'single malt', and that all the cows in a given field tend to face in the same direction. This last is true at least for the fields along Interstate 80 between Pennsylvania and Wyoming. These states are the twin poles around which Krishtalka's life revolves, because he curates bones in the former but unearths them in the latter. Anecdotes drawn from Krishtalka's personal experience riddle the book, and although they add life to an isolated essay, their cumulative effect in a collection such as this may be cloying for some. Nevertheless, the western view of the domestication of the cow is both entertaining and informative, especially the idea for employing the monolithically orientated cattle as billboards along the highway.

The range of topics is eclectic, not to say eccentric, but is not far from what one might find in an Asimov collection. Intelligent life in the Universe, DNA, fossils (lots of them) and broadsides against creationists, spiritualists and other hangers-on all get a look in. But there the similarities end. Krishtalka's informal style is a world away from The Good Doctor's eastern polish, and when sounding off on things he feels strongly about his is the wry and intimate voice of the saloon bar regular rather than the insistent tone of a soapbox orator. Here is a book to save for the next time you're lonesome on the trail. □

Henry Gee is on the editorial staff of *Nature*.



# The boy from Missouri

Alan J. Charig

**Digging into the Past: An Autobiography.** By Edwin H. Colbert. *Dembner Books*, New York: 1989. Pp. 456. Distributed by W.W. Norton, \$25.

PERHAPS I am not an altogether suitable person to review Edwin Colbert's autobiography. As a vertebrate palaeontologist myself (indeed, his transatlantic 'opposite number') and a personal friend, I have revolved in many of the same circles as he; it is therefore only natural that I should show an exceptionally strong interest in the people, the places, the events and the subjects of which he writes. Nevertheless I cannot imagine that anyone could fail to be absorbed by this delightful book.

The author is a very ordinary man, in the best sense of that word. He has a wide diversity of interests and pleasures: dinosaurs, birds, Indians, travel, gardening, baseball, limericks — to name but a few. Modest and unpretentious, he sees himself as no different from anyone else. Yet, paradoxically, he is also quite extraordinary. In his chosen profession he has

reached the very summit, for he has been (simultaneously) a collector of vertebrate fossils from every continent, a senior curator of one of the world's leading natural history museums, and a researcher and prolific writer of scientific articles. His family life — first as son, then as husband and father of five boys, and more recently as grandfather — has likewise been remarkably happy. It is clear that Ned Colbert, both in his career and in his private life, has achieved fulfilment; such contentment, today, is a rare condition, and must surely be the greatest prize of all. If his book has a flaw, it is that its author looks at the world through rose-coloured spectacles; he sees

little fault in anyone (except the pompous, imperious Professor Osborn, self-appointed doyen of American vertebrate palaeontologists in the days of Colbert's youth). Indeed, nearly everybody and everything are "nice", which goes only to show how nice is the author himself.

Colbert has written many highly esteemed semi-popular scientific works. They are complemented by this new book, which is more personal, without too much of a technical nature; a palaeontological paragraph appears occasionally, but only to explain the broad divisions of geological time or of vertebrate life or, more often, the purpose of a particular visit or expedition. Rather, the book is an historical account of Colbert's own life, introducing *en passant* a series of cameos of people — mostly 'characters' — and things. Ned has a fantastic memory for detail; thus the house in which he spent his childhood in rural Missouri is lovingly described. Much of the detail is trivial, but it is always amusing. The cameos are inconsequential, not in any pejorative sense but in the sense that there is little connection between them (other than that of chronology). In each chapter, however, the individual items together build up, not just a picture of the author's own life, but also an impression of, say, life in a small mid-Western town in the first two decades of this century, or what it was like to be a member of the New York academic scene between the wars, or (more recently) of an expedition to Antarctica.

We begin with Colbert's birth in Iowa in 1905, his family history, the house in Maryville, Missouri, and his boyhood in that town, omitting neither his pranks nor his beloved dogs. His introduction to the world of nature and a brief interlude as a trainee forest ranger were followed by an awakening interest in palaeontology; that,



Colbert (right) on expedition in Antarctica in 1969.

From Digging into the Past

in turn, led to an apprenticeship at the university museum in Lincoln (Nebraska) and then to Columbia University in New York. Columbia had close links with the American Museum of Natural History, and it was at those two institutions — especially the latter — that Colbert spent the greater part of his professional life. From then on we are treated to an entertaining and informative account of his career. He describes at length the places he travelled to and worked in, both in the United States and abroad; the people he met (most of them amusingly eccentric); and, less frequently, the fossils he dug up. The book ends with a chapter on museums, another on dinosaurs, and the last on his retirement years, spent with his wife Margaret in the beautiful surroundings of Flagstaff, Arizona. Their house, of course, is next door to the local museum, where Ned still spends much of his time.

Each of the 22 chapters is prefaced by a charming black-and-white drawing by his wife, appropriate to the title of that chapter — be it a somewhat apprehensive young Colbert sliding down the hillside on his sled, or a Triassic gliding lizard, or a gulch on the edge of a Brazilian village. There are also 32 pages of black-and-white photographs, and an excellent index.

Ned Colbert wrote in his obituary of Alfred Romer (a slightly older, equally famous, equally lovable American colleague) that "he took his paleontology seriously but never himself". Colbert's autobiography shows that this compliment is just as true of himself. □

*Until his retirement Alan J. Charig was Chief Curator of Fossil Amphibians, Reptiles and Birds at the British Museum (Natural History), Cromwell Road, London SW7 5BD, UK, where he is now a Research Associate.*

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# Archaeological cross-talk

Richard Bradley

**In Search of the Indo-Europeans: Language, Archaeology and Myth.** By J.P. Mallory. Thames & Hudson: 1989. Pp.288. £24, \$29.95.

PREHISTORIANS make a virtue of a necessity. Deprived of written evidence, with all its latent biases, they have devoted enormous efforts to devising methods of talking about the past. The ideas may be adapted from those of anthropologists, of geographers and, sometimes, of historians, but the raw material for their studies comes from the purely physical by-products of human behaviour. Only occasionally were they intended to convey information. Otherwise the work of archaeologists depends on understanding the relationship between human history and the peculiar character of the material that they investigate.

The methods of obtaining that evidence may be eminently practical, yet archaeologists cannot be innocent of theory once they come to interpret it. But theories are notoriously volatile. From an overriding concern with the chronology and distribution of objects, archaeology has come to investigate the changing character of society itself, and accounts that had long coloured popular understanding of the past have been found wanting. The most fundamental assumption to be questioned is that the more important changes in prehistoric Europe came about through the movement of peoples. Such an assumption implies a quite specific sequence of events, and sometimes this expectation has been confounded by scientific dating. In other cases archaeologists have been influenced by work in social anthropology and have come to understand how styles of object and systems of belief may be adopted over enormous distances in order to emphasize the position of local élites. The result is a much greater emphasis on local factors and on the whole process of social evolution.

The lack of written sources has given archaeologists a creative freedom, derided or envied by those who work in historical periods. Indeed, there are mediaevalists who believe that the archaeological record should be given priority over the more partial evidence of documents. This is a curious position, for by working with two different types of evidence historical archaeologists should be able to shed light on the strengths and weaknesses of the subject as a whole. For the prehistorian there is one comparable challenge to be faced. We know that by the dawn of written history societies as far apart as Ireland and Iran shared those closely related languages grouped together

as 'Indo-European'. How and when did these relationships develop? It is here that archaeology must come to terms with a less familiar kind of evidence.

Prehistoric archaeology bears the imprint of one great man, Gordon Childe, who died over 30 years ago. Childe's achievement was to establish the basic grammar of the subject and to define the broad character of prehistoric Europe, using an approach influenced by his reading of Marx. His interpretation presupposed the movement of peoples and ideas from east to west, and from civilizations



British Museum

Archaeological evidence or just local colour? — a Siberian belt plaque, 5th–4th century bc.

into their barbarian peripheries. It is this part of his interpretation that has fallen victim to radiocarbon dating. But it is sometimes forgotten that Childe was also a linguist, profoundly influenced by his understanding of the geographical and chronological spread of ancient languages. One of his first books was entitled *The Aryans* (Keegan Paul, Trench, Trubner and Co., 1926), another name for the Indo-Europeans. It was precisely because the study of past languages became so deeply implicated in Nazi racial theory that Childe discontinued this side of his work. As a result of similar inhibitions, few people have confronted the relationship between archaeology and language.

Things are changing. Two years ago Colin Renfrew published a controversial study of this question (*Archaeology and Language: The Puzzle of Indo-European Origins*; Cape/Cambridge University Press, 1987); now Dr Mallory has produced an interpretation which differs fundamentally from that account. The issues have been keenly contested, with

special numbers of leading journals being devoted to the theme (one protagonist in the debate has reviewed Renfrew's book, in increasingly heated terms, on at least three occasions). It is not hard to see why such a technical discussion should arouse so much interest, for it epitomizes the differences between two distinct attitudes to our study of the past.

For the 'traditionalists', among them Dr Mallory, the linguistic issues must take priority over the archaeological record. There are certain fundamental principles to do with the ways in which languages spread and the rates at which they change, and these cannot be challenged. Ultimately, the archaeological record must be consistent with those types of evidence: it cannot be decisive in itself and must accommodate the findings of the linguists. That means that we must postulate migrations for which the archaeological evidence alone is rather limited. For

Renfrew, on the other hand, archaeologists have gained sufficient confidence in handling their material that such a position is untenable. If linguistic theory does not measure up to the archaeological record, its own premises need to be reviewed. He identifies a fascinating failure of communication, in which both parties have deferred to one another — the linguists accommodating their analysis to what Renfrew considers an obsolete reading of the archaeology, and the archaeologists assuming that linguistic orthodoxies have to be taken at face value. The debate, then, is about the success or failure of archaeological interpretation itself, and Mallory and Renfrew are ranged on opposite sides.

The two historical narratives differ in fundamental ways. Mallory follows the traditional interpretation that the Indo-European languages spread through the migration of peoples from a homeland in the southern part of the Soviet Union and that this took place from the late fourth millennium bc onwards. The migrants were mainly pastoralists; they had a



distinctive social system, echoed in our earliest written sources, and a series of religious beliefs that underpin the literature of many different parts of the world. For Renfrew, on the other hand, the archaeological evidence must be paramount, and he can find none to support a large-scale migration at that time. The main changes in the archaeological record are better explained in terms of local social developments. That is why he considers that the Indo-European dispersal must have taken place at a much earlier time. The only period in which such large-scale expansion is seen in the archaeological record is with the spread of mixed farming around 6000 BC. Renfrew's Indo-Europeans originate in present-day Turkey. He develops a series of models to help us to understand how and why languages might have changed their distributions, but for Mallory these are problems that have already been solved by the linguist. For that reason, archaeology plays a subsidiary role in his discussion.

I have stressed the importance of the debate and the extent to which the participants disagree. That is because the point at issue seems to be the validity of the very methods used by archaeologists today. Were they right to reject the approach taken by Gordon Childe, with its emphasis on the movement of peoples? In the absence of other sources of information, such as the linguistic evidence discussed in great detail by Mallory, how can they decide between competing interpretations? It is the merit of this book that it states its position so clearly.

*In Search of the Indo-Europeans* lacks the rhetorical drive of Renfrew's more polemical statement, but in its place offers a detailed and fully illustrated account of the linguistic issues, and reviews the relevant archaeological evidence. It is well organized and well documented, but the reader can easily get lost in the archaeology. This is a pity because much of the material is unfamiliar and bears witness to Dr Mallory's extremely wide reading. On the other hand, it is the earlier chapters, where Mallory is concerned with broad issues, that work best on the page. His account of the linguistic evidence is actually more persuasive than his presentation of the archaeological sequence; indeed, some of the most telling arguments are to be found in the notes, and it is here that his differences with Renfrew come into sharpest focus.

Both books present a persuasive argument, but both cannot be correct. So how are we to proceed? If we concede the primacy of the linguistic analysis, the archaeological material is relegated to a supporting narrative, supplying local colour but insufficient to test the basic hypotheses. Mallory takes rather this line, rejecting the claims of theoretical archaeology to provide a self-sufficient inter-

pretation of the past. If recent archaeological research is correct, however, the linguists must think again.

Can we find other sources of information that may help to break the deadlock? Perhaps we should look more closely at research on the distribution of human blood groups, and even at the evidence of population change provided by modern physical anthropology. We may be inhibited by the ways in which such studies have been turned to political ends, but there seems to be little alternative. An impor-

tant debate is taking place, immeasurably sharpened by the clarity with which the rival positions have been expressed, but that discussion threatens to take on a hermetic quality and must be broadened. In the meantime I have every praise for Dr Mallory in expounding what may seem an unfashionable view, and for doing so with such lucidity, balance and good humour. □

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## In those days

Warwick Bray

**The Pastmasters: Eleven Modern Pioneers of Archaeology.** Edited by Glyn Daniel and Christopher Chippindale. *Thames & Hudson: 1989. Pp.176. £18, \$22.50.*

THE essays in this book were commissioned by Glyn Daniel and published separately in *Antiquity*, all but one of them between 1980 and 1989. Eminent archaeologists were asked to look back over their careers, to summarize what they thought were their achievements (and sometimes failures), and to discuss what personal and intellectual influences had moulded their lives and personalities.

The chosen few, all men, are Childe, Piggott, Phillips, Hawkes, Seton Lloyd, Braidwood, Willey, C. J. Becker, De Laet, Desmond Clark and D.J. Mulvaney. There is a touch of cronyism in the selection; these are people Glyn Daniel knew and liked, but each of them is a figure of real substance. Some — Clark in Africa, Mulvaney in Australia — span the entire history of archaeology in their respective areas. Some are field men, others synthesizers and teachers, but all of them, through a mixture of scholarship, administration and patronage, helped to form archaeology as it is today. The Americas are under-represented, but it is good to see the intellectual contribution of Scandinavia and the Low Countries receiving due recognition.

Each author has produced what was required, a sort of anecdotal curriculum vitae rather than a critique of archaeology as it is now. Some of the stories confirm the popular impression of the protagonists. The shocking state of Gordon Childe's trousers after 25 years of hard wear will surprise nobody, nor will the pedigree of Stuart Piggott (a chalkland hobbit cruelly uprooted to Scotland), whose family "had been around in Marcham, Hatford and West Challow since the early seventeenth century". Less predictably, Willey was a would-be track star, and Clark believes the teamwork of a

rowing eight is ideal training for teamwork in the field. These trivia are entertaining, but they also help to reveal the personalities behind the publications (and the fate of scientific programmes, individual careers and university departments has as often been decided by personality as by reason and justice). So it matters that Christopher Hawkes, in his otherwise solemn essay, wants us to know that he danced an exhibition tango at the Mediterranean Congress in 1950. My reaction to this is summed up by van Giffen's remark at the time: "I did not know you were such a man".

The papers gain from being printed together and, cumulatively, they give an inside impression of the archaeological world at the critical time when it was first becoming professional. It is a picture I recognize from my own student years. The world was smaller and quieter then. There was less to know; everybody knew everyone else; whole new continents were opening up to archaeology, and radio-carbon dating was beginning to sort out the muddles over chronology. There were pressures, too, and occasional unpleasantnesses, but these are discreetly glossed over in this rather bland book.

Members of the 'Don't trust anyone over 30' generation often complain that their elders were naive in matters of theory, and far too preoccupied with digging, dating and writing culture history. These people should read the eight-page contribution by Gordon Childe, written in 1958. He was wrong about many things, but he had absorbed the works of Spengler and Hegel, Malinowski and Durkheim, as well as Morgan and Marx. He drew upon the philosophy of science (and of history), employed ethnographic analogies, worried about modes of production and the nature of culture, and sought laws of human behaviour analogous to those of physics and chemistry. Childe, and several of the others in the book, were as innovative in their time as any of today's Young Turks, and it does no harm to remember that once in a while.

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# Nothing but the facts?

Jeremy A. Sabloff

**What Is Archaeology? An Essay on the Nature of Archaeological Research.** By Paul Courbin. Translated by Paul Bahn. University of Chicago Press: 1988. Pp.197. \$24.95, £19.95.

ARCHAEOLOGISTS who enjoy watching William F. Buckley skewer a liberal guest on the American television programme *Firing Line*, or relished Malcolm Muggeridge making mincemeat of a pseudointellectual, are certain to have a great time reading Paul Courbin's book. *What is Archaeology?*, which was originally published in 1982 in French, is a short, harsh attack on the 'new archaeology' of Lewis R. Binford, David Clarke and many others who have tried to improve archaeological knowledge over the past three decades. I savoured a good polemic as much as anyone, yet when I finished the book I was left with the same feeling that most of us have when we have eaten a plate of junk food. It's been fun, but one can have too much of it.

For many scholars, the nature of archaeological research has changed dramatically, albeit sometimes painfully,

## The New World of archaeology

IN HIS review of *The Pastmasters* (facing page) Warwick Bray voices the mild complaint that the Americas are under-represented in the collection. Partially compensating for that deficiency is a similar kind of book, *Portraits in American Archaeology: Remembrances of Some Distinguished Americanists*, to be published on 28 April by University of New Mexico Press (price hbk \$35, pbk \$19.95).

Unlike the essays in *The Pastmasters*, all of the portraits are a product of a single hand, that of Gordon R. Willey. But both books reflect earlier times when archaeology was very different — less 'scientific' but, on Willey's evidence, rather more pleasurable to work in, because only a small fraternity was involved. As Willey writes in his preface, of when he was starting out on his career in 1937: "the annual meeting of the Society for American Archaeology . . . was held in a single room and with single sessions. Attendance was probably on the order of 30 or 40 of us, young and old. . . . We were terribly dependent upon each other".

There are 16 portraits altogether, an avowedly personal selection of people Willey knew and liked. This is no reference work; but it is well-written and enlightening, and imbued with the author's affection for his subject.

T.L.

since the early 1960s. Archaeologists now try to understand long-term cultural changes and have developed innovative techniques and methods to reach this goal. Both the planning of research and the interpretation of research results have become much more rigorous. Other tenets of the new archaeology, such as the necessity of a deductive-nomological approach to establish archaeological laws, appear to have failed to take hold.

Courbin, a professor of archaeology at the Ecole des Hautes Etudes en Sciences Sociales in Paris, denies that any significant changes have taken place. He zeroes in on the excesses of the new archaeology with scathing sarcasm that — in Paul Bahn's effective translation — dissects the jargon-laden claims of many writers with great dexterity. But Courbin is so blinded by outrage that he loses sight of several of the new archaeology's genuine accomplishments.

Although Courbin has been assiduous in reviewing the theoretical or programmatic literature, he does not pay equal attention to the literature documenting fieldwork. Why, just to give a few examples from the New World, is there no mention of Howard D. Winters' path-breaking *The Riverton Culture* (Illinois State Museum, 1969) or of the reanalyses undertaken by Stuart Struever and others of the Hopewell culture? Why is a diagram from David Hurst Thomas's Great Basin research reproduced but the innovative fieldwork ignored? Why, unless Courbin considers it traditional archaeology, is there no citation of Kent V. Flannery's important *The Mesoamerican Village* (Academic Press, 1976)? Why, in the text itself or in his new introduction to this English edition, does he not recognize the growth and development of Binford's thinking or the changes of emphasis in the work of Patty Jo Watson and her colleagues (compare *Explanation in Archaeology*, published by Columbia University Press in 1971, with *Archeological Explanation*, issued by the same publisher in 1984)?

Courbin would have the reader believe that the new archaeology has failed and that traditional archaeology triumphantly continues on. This contention is not correct. Although many of the specific proposals of the 1960s and early 1970s have not been followed, the practice of archaeology in the New World and elsewhere has been profoundly affected by fresh ideas.

In my view, the most notable changes have been methodological in nature, not theoretical. Courbin is correct when he argues that there has been relatively little progress in theoretical understandings of long-term change. But he does not acknowledge that much effort has gone into developing methodologies that have the potential to lead to new understandings. Certainly, there is no evidence

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whatsoever that the methodologies of traditional archaeologists were leading to any advances along these lines. Even the old boasian belief, that 'when the facts are in, new theoretical understandings will emerge', eventually gave way in American anthropological thinking to the realization that that great day would never arrive.

Of particular relevance to methodology was the argument by Binford and others that culture is variable, not homogeneous. From this premise, it follows that archaeologists need to cope with variability in as rigorous and systematic a fashion as possible. Such thinking implied that overall research strategies and the means by which variability could be recognized in the archaeological record had to change. Problems of sampling became paramount in fieldwork and highlighted the need to give explicit attention to the ways in which meaning was routinely assigned to various patterns in that record. This argument cannot be dismissed by those such as Courbin who say that archaeologists are interested first and foremost in gathering 'facts'. What kinds of facts are they collecting and how useful will they be if sampling problems are ignored? New perspectives on the Maya, for instance, have shown how traditional views focused only on elite aspects of the civilization and missed much of the rest of activity in that society.

So Courbin's contention that traditional archaeologists can continue with business as usual, at least in the Americas, is disingenuous at best. To my mind, what he is calling for is for the clocks to be turned back to the pre-1960s. But as David Clarke so insightfully argued in his article "Loss of Innocence" (*Antiquity* 47, 6-18; 1973) — an important paper not discussed by Courbin — once archaeologists' eyes have been opened to the critical role their biases and perspectives play in uncovering and interpreting data, they cannot close them again. Thus I would argue that archaeology should not be limited to the 'establishment of facts'. Courbin's separation of the archaeologist as archaeologist recovering and analysing facts, and the archaeologist as anthropologist or historian interpreting them, is hopelessly naive.

Courbin acknowledges that there are no archaeological facts waiting to be discovered. He understands that archaeologists bring "frames of reference" (to use his term) to their examination of the archaeological record and that some degree of interpretation is necessary in the establishment of facts. Archaeologists, for example, usually do not discover perishable houses, but instead uncover circles of stone that they interpret by analogy as dwellings. It is in this critical area that Courbin is at his weakest. He seems confused, for instance, when he says (p.122):

Finally, I can reassure those who might think it humbling to devote oneself to the establish-

ment of archaeological facts. This is not as unworthy of their immense talents as it might seem. If truth be told, it is not easy; it is an enterprise that is at least as difficult as, and perhaps more so than, the invention and demonstration of a law (or would-be law) or the building of a theory, be it "middle-range" or general. . . .

Courbin does not seem to realize that middle-range theory helps the archaeologist bridge the gap between the static archaeological record and all kinds of interpretation, which often include the traditional archaeologist's 'facts'. Although Courbin recognizes that facts involve interpretation, and that establishing them is often a difficult procedure, he mistakenly insists on separating the activity of establishing facts as purely archaeological and the further interpretation of them as something else that archaeologists do that is anthropological or historical but not archaeological. In the process of assigning meaning to the data on the ground — in their reporting of facts — archaeologists function as anthropologists or historians, whether such actions are explicit or implicit.

In addition, Courbin's dismissal of materialism as deterministic is too simplistic and not well argued. He falls into the same trap that he derides the new archaeologists for falling into; namely, labelling an argument and assuming that the label itself is sufficient refutation. As Leslie White, some years ago, and Marvin Harris, among others, have demonstrated, being a materialist (or being ecologically orientated) does not mean that one does not believe in personal liberty.

*What is Archaeology?* is definitely worth reading. Archaeologists and knowledgeable scholars from neighbouring disciplines will find that it will help sharpen their thinking about modern directions in archaeological thought. Like most polemical tracts, it will either have you cheering or have your blood boiling — Courbin takes no prisoners. But students or others unfamiliar with the controversy over the new archaeology should read it in conjunction with other books, for example Lewis R. Binford's *In Pursuit of the Past* (Thames and Hudson, 1983), Ian Hodder's *Reading the Past* (Cambridge University Press, 1986) or the second edition of the late Glyn Daniel's *Idea of Prehistory* (Edinburgh University Press, 1988), paying particular attention to Colin Renfrew's two new, informative chapters. I believe that such comparative reading will convince the bystander that Courbin's critique misses the mark. Collecting 'facts' is not a sufficient rationale for archaeology — the discipline is and can be much more. □

Jeremy A. Sabloff is University Professor of Anthropology and the History and Philosophy of Science at the University of Pittsburgh, Pennsylvania 15260, USA.

# Science as she is wrote

Walter Gratzer

**How to Write & Publish a Scientific Paper, 3rd edn.** By Robert A. Day. Oryx Press, 2214 N. Central at Encanto, Phoenix, Arizona 85004/Cambridge University Press, UK: 1989. Pp.244. Hbk \$23.50, £20; pbk \$16.95, £7.95.

**The Technical Writer's Handbook: Writing with Style and Clarity.** By Matt Young. University Science Books, 20 Edgehill Road, Mill Valley, California 94941: 1989. Pp.256. \$22.50. To be published in Britain by Oxford University Press, £14.95.

TECHNICAL literature may be short on charm, but the discriminating reader will often find in it examples of writing so bad that it glows with a kind of grandeur all its own. Let me offer you a humble specimen, culled from the letter page of the *Times*. A puzzled customer, inquiring of a biscuit manufacturer why the tin containing his products was not quite square, was rewarded by the following reply:

The existing sizes were developed because prior to the war, a much larger range of sizes was offered.

Small tins were then available which were known as no 1 and no 2 size tins, and for stacking purposes four no 1 size tins equalled in size the half square biscuit tin. Eight no 1 tins were equal in size to a square tin and, similarly, four number 2 tins were equal in size to a square tin.

These very conveniently fitted both racks and storage spaces and we are afraid that any alteration of the size would now cause considerable inconvenience to the trade.

Does this not have a certain majesty? Or consider the ringing tones in which a circular letter from the Monsanto Company apostrophizes its reader: "Dear Friend of Maleic Anhydride".

Small wonder then that technical writing has become identified as a distinct literary genre, which attracts its own apologists and indeed scholars, who profess it as an academic discipline. One such is Robert A. Day, whose book, now in its third edition, is evidently the outcome (jokes and all) of a series of lectures at the University of Delaware. Professor Day is an enthusiast for his subject and he is generous, if not profligate, with information and advice, down to the exhortation that when your paper is at last written you must "make sure that you apply sufficient postage and that you send the package by first-class mail". And mark that before you speed it on its way you would do well to have it scrutinized not merely by the other authors but by a small team of scholars, to include "(a) a scientist working in your field, (b) a scientist working in an unrelated field, and (c) a competent grammarian [eh?]. Careful management of this presubmission process is likely to improve the chances of acceptance by the journal".

There, I fear, speaks the whilom managing editor of the *Journal of Bacteriology*, viewing the tribulations of the

laboratory scientist with detachment. The medium has become the message.

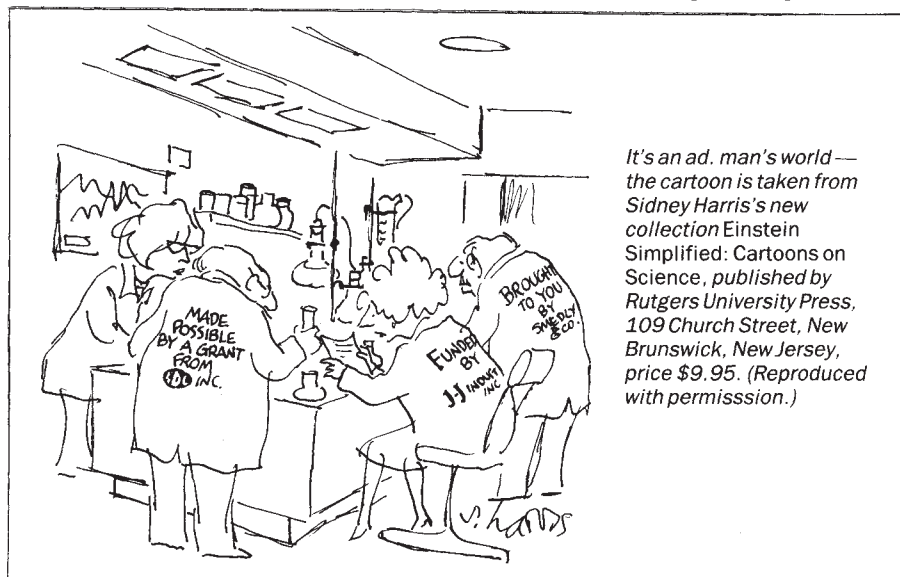
There is still another recurring theme in Professor Day's discourse, which made me sometimes wonder whose side he is really on. He seems to me to be altogether too concerned that we should give no displeasure to members of the bossy professions — to copy-editors for instance, who deny us the correct use of hyphen or gerund; especially must we obey those exigent lice in the armpits of science, the nomenclature commissions, when they forbid us to utter words such as calorie or molecular weight.

But then perhaps Professor Day is right to insist that if you heed his admonitions you will improve your chances of getting your papers into the journals in which the best people disport themselves. 'Prestigious' is a word much favoured in this context. ("Prestigious, *a.* Now rare. Practising juggling or legerdemain . . . cheating, deluding, deceitful; deceptive, illusory" — *OED*.) When you have identified these journals, which you do by looking up the outstanding papers in your field, you will know where to direct your offering. But, please sir, what if my research is tedious, trivial and derivative (like most other people's)? Will the same advice then serve for me as for the Nobel laureate down the corridor? Surely my problem will be less a matter of placating the copy-editor with my faultless use of

units and abbreviations than how best to gloss over the frailty of my logic, disarm the referees and construct the figures so as to flatter the data.

Perhaps I have cavilled too much, because after all Professor Day's main principles are sound. Besides extolling brevity and preferring statistics to be meaningful, he is (as I judge) consistently on the right side on such matters as declamatory titles (hostages to fortune, if nothing else; think for instance of "Space Travel is Utter Bilge" from the Astronomer Royal a year or two before Sputnik I), and much more. Both Day and Matt Young — whose handbook begins with a mere dozen pages of cogent general advice on style in technical writing — work themselves into a fine lather on hanging participles (striding into the laboratory he saw a white rabbit), which they would clearly run a mile in tight shoes to escape; each delivers more than one philippic about the superiority of the active over the passive voice, and the first over the third person. For my part, I am irked by the too frequent intrusion of the author (we weighed the sample and then we dissolved it in water); and you will find in an earlier volume of *Nature* an illustration of the over-zealous application of such canons. A book-reviewer's flattering observation, "the author is of course the world's foremost authority on the genetics of . . ." was transformed by creative copy-editing into "I am of course the world's foremost authority . . .". This shows that a man must always retain the capacity to rise above principle (or that copy-editors too are responsible for the sense of the copy).

Of the two books, Matt Young's is the more ambitious. Where Day is concerned largely with the mechanics of assembling publications and getting them into print, Young has compiled a dictionary of technical English usage, rather on the lines of Fowler's *Modern English Usage*. He has a



It's an ad. man's world — the cartoon is taken from Sidney Harris's new collection *Einstein Simplified: Cartoons on Science*, published by Rutgers University Press, 109 Church Street, New Brunswick, New Jersey, price \$9.95. (Reproduced with permission.)



lively and informed interest in the language and its development, and a sharp eye for the manifold abuses that are heaped on it in the technical prose of our day. He warns for instance against 'verbi-fication', as he calls it ('to formularize' is a grisly example), but he endorses 'key-boarding' (as does Day). Those of us who have never boarded a key may console ourselves with many classical examples of what can happen when nouns and verbs get confused. Consider for instance the celebrated wartime headline EIGHTH ARMY PUSH BOTTLES UP GER-MANS (which also has a lesson concerning nouns of multitude). Young sets up what seems to me to be a number of straw men: do we really harbour in our profession people who say "few number", or "to downsize" or "volunteerism"? He excoriates analogue (instead of analog), catalogue and dialogue as archaic, and he asserts that collective nouns take the singular in America but the plural in England; thus Congress is in session, but Parliament are in session. Well are it? Fowler, at least, lays down no rules for 'nouns of multitude', and says we may use whichever sounds more euphonious.

Young has allowed some solecisms to slip through. He does not care for "Neither Randi, Maddox, or Stewart has a background in immunology"; you should write "neither Randi, Maddox, nor Stewart" has such a background. But surely 'neither' can refer only to one of two things, not of three. Sometimes the solecism is calculated, as when Young urges that we should do away with 'whom' (except after prepositions) because, he says, it is hardly ever used correctly. Yet this is not a new problem — compare "Whom are you", he said, for he had been to night-school", from a novel of 60 years ago — and Young's solution dismays me. He also believes that it is time to abandon the distinction between 'shall' and 'will', and it is true that there occur in the American literature sentences on the lines of: "this technique shall be the method of choice". Fowler encapsulates the distinction in the cries of the victim ("I shall drown and no one will save me") and of the suicide ("I will drown and no one shall save me").

Elsewhere also Young takes a confessedly permissive line. He writes judiciously about hyphens, but in the end is content to let the journal be the arbiter, and, as he observes, the trend seems to be to extirpate the hyphen altogether, with further sacrifice in precision. Fowler's exemplar of ambiguity is "the hard working man"; newspaper headlines are ever a good source — HORSE BACK UP SNOWDON is one that I recall. So 'save the hyphen' should have been Young's plea (not that even hyphens are proof against misreading: another headline, this one from the Korean war, went

MACARTHUR FLIES BACK TO FRONT. Here it seems as though the imagination supplies the phantom hyphen).

Well, I have done. But I find that I have not obeyed Professor Day's injunctions in his chapter "How to Write a Book Review". It is just that I seem to be unable to decide whether it is a pair of monographs, reference books, textbooks or trade books that I have been considering (for these must be assessed by different criteria). No matter, for in all cases my brief is to ensure

## Sally on trial

J.A. Gray

**The Unheeded Cry: Animal Consciousness, Animal Pain and Science.** By Bernard E. Rollin. Oxford University Press: 1989. Pp.320. £17.50. To be published in the United States in June, \$29.95.

BERNARD Rollin hopes that his book will "help scientists break the ideological bonds which keep them from ascribing mental states to animals", and so free them to hear the "unheeded cry" of its title — the cry of laboratory animals. By the end of the book, I at any rate had come to feel that Rollin's heart and mind are both in the right place, as he claims that:

Moral theory and a science of all animal consciousness . . . will inevitably stand in a dialectical relationship, for the burgeoning questions regarding the moral status of animals lead to a variety of questions about animal awareness which science must try to answer, and the study of animal awareness in turn generates its own moral questions.

Here is a promising programme of work — capitalizing on the new moral awareness, in large measure due, as Rollin rightly claims, to the animal welfare movement, but not threatening the whole research enterprise. But, had I not agreed to review the book, I doubt that I would have read as far as this sane and forward-looking conclusion.

To begin with, Rollin's softly-softly message is not helped by the rhetoric in Jane Goodall's foreword ("atrocities . . . perpetrated behind the closed doors of underground animal research laboratories around the world"). Even less is it helped by the straw scientist he sets up as his Aunt Sally. This person is supposed to have accepted an 'official' scientific orthodoxy, according to which animals experience no feelings, and especially no pain, so it doesn't matter what one does to them in the name of science. This orthodoxy has been foisted upon our straw scientist by a conspiracy of psychologists, the chief devils being Watson, Lashley, Skinner and Hull (yes, it is time to kick the behaviourists again). Challenged with the

that "a potential reader will know whether or not to read the book under consideration and why". So yes, the Potential Reader should read these books, if he has time; but not before he has read Fowler. And the reason why he should read them is that if he follows the precepts set out in both he will write shorter, clearer (and, who knows, fewer) papers, and that will be all to the good. □

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oddity of his beliefs, our scientist retreats into moral confusion and incoherent recitation of his creed ('science is value-free', 'all I want is the facts', 'keep philosophers out of the laboratory'). But hope is at hand: scientific credos are largely a matter of fashion, anyway, and times are changing, thanks to those splendid fellows the 'animal activists'. So it won't be long before we all accept again — as common-sense did all along — that animals have feelings, and stop committing atrocities.

This caricature is constructed almost entirely from anecdotes (Rollin appears to attend a large number of dinner parties). Far from being apologetic, Rollin prides himself upon this: "like Hegel, I am a believer in . . . particular cases which vividly instantiate and communicate a general truth". So I shall make no apology for answering anecdote with personal history. After all, as a psychologist working on the brain, I am just the kind of guy that Rollin's straw should be stuffing. But his anecdotes barely begin to fit. It has never occurred to me to doubt that animals experience pain. Why else would I use anaesthetics during surgery? (According to Rollin, the official answer to this question is "for chemical restraint".) Nor do I doubt that animals experience other feelings: on the contrary, it is precisely in order to understand the nature of feelings such as anxiety that I work with them. It is, I believe, possible to do this neither immorally, nor in a state of moral confusion, but trying to minimize the harm done to the subjects of the experiments while maximizing the good that comes to people, in the form of both knowledge and medical advance. (This is known, in Peter Singer's phrase, as "speciesism", a charge I am not ashamed to accept.) That is what the reality of hard moral choices is about.

To be sure, some — a very few — psychologists espoused a radical form of behaviourism, according to which conscious experience is simply a fiction. I remember asking one such person to tell me the difference between what happens when a Mozart string quartet is played to a hearing man and to a deaf man; his unconvincing answer turned upon the verbal behaviour they would emit under appropriate circumstances later. But, for most

of us, behaviourism has always been a methodological principle. Behaviour (or the workings of the brain) is all that you can observe (and neither Rollin nor anyone else has yet come up with any viable alternatives); the rest is theory. And, as a methodological principle, behaviourism says nothing about the existence or otherwise of feelings. But what behaviourism has done, and Rollin signally fails to recognize, is enormously to raise the standards of evidence required for the ascription of feelings or other psychological constructs to animals; and it is this rise in standards that makes the research programme he adumbrates at the end of his book realistic.

The trouble with anecdotes is that there are no rules for choosing between them (though Rollin devotes much space to Romanes' suggestions for such rules). But, apart from the protagonist in the Mozart story, I have never met a scientist who does not believe in the reality of pain and other feelings in animals (which is not to deny the formidable obstacles to their empirical study). It seems likely, then, that Rollin's book will fail in its stated aim: if the scientists who experiment with animals already believe them to have feelings, then convincing them further of this fact will be a poor way to persuade them to desist. So what does the book achieve? It contains a useful review of pre-behaviourist attempts to theorize about animal behaviour (Romanes, Lloyd Morgan, Loeb, Jennings); some examples of the influence of social and ethical factors upon scientific beliefs (though these do not show — as Rollins sometimes seems to argue — that the truth of these beliefs depends upon such factors); and a valuable personal account of recent shifts in scientists' moral concerns about animals.

Sadly, however, the book may also achieve something more sinister. One man's anecdote is another man's smear. The picture Rollin paints of scientists is not flattering (though some of his best friends, it seems, are scientists). We are motivated in the main by ambition and career; scientific fraud is rife; we anaesthetize our conscience by the myth that animals have no feelings; and (according to one after-dinner chat) the only reason we don't do our awful experiments on children is "because they won't let us". Rollin complains that a previous article of his was criticized as providing "a moral ground for laboratory break ins". He should have pondered this criticism more seriously. The distance between the smear and the break-in — or more dangerously, the bomb — gets shorter all the time. It would have been a pleasant surprise, in such a book as this, to see an unequivocal condemnation of such violence. Alas, it is not there. □

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## Half a century of fear and of peace

John Maddox

**Danger and Survival: Choices About the Bomb in the First Fifty Years.** By McGeorge Bundy. Random House: 1989. Pp.735. \$24.95.

McGEORGE BUNDY, once one of Harvard's youngest-ever Deans of Arts and Sciences, was translated in 1960 to Washington with and by John F. Kennedy, and promptly became part of the then liberal demonology. With a background in the international control of nuclear weapons, he became one of Kennedy's hard men, responsible for defining and then making clear the reasons why the government of the United States reacted with such force to the Berlin blockade (in 1961) and the Cuban missile crisis two years later. On the evidence of his book, Bundy's main difficulty may have been that he writes too clearly for what he means to be misunderstood.

Bundy is not of course a fully fledged hawk, as has been clear from his spell as President of the Ford Foundation and, now, as a part-time academic in New York and at the Massachusetts Institute of Technology. *Danger and Survival: Choices About the Bomb in the First Fifty Years* is about the personal and political consequences of nuclear weapons. Despite its bulk, it is a gripping tale of the incorrigible failure of the human imagination to comprehend the enormity of the success of the Manhattan project, and the later developments and their consequences.

The tale of how Stimpson, Secretary of War in 1945, failed to persuade Truman that diplomatic prudence required direct discussions with the Soviet Union of the fact of US nuclear weapons, has been told before, notably by Bundy himself. Some in Washington calculated that the mere knowledge that the United States had developed a nuclear bomb would ensure Soviet compliance in negotiations on other matters, principally Central Europe, but Stimpson argued for open discussions on how nuclear weapons would change the relationship between the powers, and for an exploration of international control. It is chastening to see how later heroes such as Acheson were so indecisive at the time.

Bundy is also good on Oppenheimer, coming close to saying that the man who had made the first bombs was afterwards deceitfully framed by Lewis Strauss, the chairman of the US Atomic Energy Commission (AEC) in 1954, when Oppenheimer's security clearance was

withdrawn. That Strauss had arranged for the FBI to bug Oppenheimer's telephone conversations, even those with the lawyers representing him before AEC's review board, has been known from a biography of Strauss. Bundy provides the evidence that Strauss also fed Eisenhower a distorted and damaging account of the board's proceedings, thus making sure, when the board eventually recommended to AEC that Oppenheimer's security clearance should be withdrawn, the White House would not intervene.

My purpose is not so much to rake over old coals (however satisfying that may be), but to illustrate Bundy's theme that the recurring need to make decisions about nuclear weapons has been a constant test of character for half a century's statesmen and scientists. The losers in the Oppenheimer case were not just Oppenheimer, but Teller (who lost "friends and self-respect"), Strauss (who lost a better job at the hands of the US Senate much as Mr John Tower did earlier this year) and, importantly, Eisenhower himself: Bundy's Eisenhower shares Oppenheimer's sense of the danger of nuclear weapons.

Bundy was at the White House during the Cuban crisis; even now, the tale he tells is spell-binding. But was it wise to have risked nuclear war without first trying diplomacy, and while giving European allies almost no opportunity to protest? Bundy (who confesses to have leant towards an air strike, not a naval quarantine) carefully considers the options only to dismiss them. What if the outcome had been different?

The other theme to be found in the book is that governments, which are hardly better placed than people to get to grips with nuclear weapons, at least work hard to comprehend them. They learned from the Cuban crisis. That, says Bundy, is the chief reason why no later crisis brought such a risk of nuclear conflict as Cuba had done.

So has it all been down (or up) hill since? Of course not, says Bundy, it's been down and then up. Even great men's strategic doctrines (McNamara's assured destruction, for example) become recipes for over-providing nuclear weapons. The purchase by third powers of nuclear independence has, in Bundy's estimation, brought neither security nor respect, but only costs.

Bundy emerges as a hawk, but one of the most temperate kind. His account of Ronald Reagan's seduction by the Strategic Defense Initiative is delicate and ironical. Nuclear weapons will continue to keep the peace, as they have done for 40 years, but the safest number is the smallest possible. Bundy's present successors at the White House should read what he has to say. □

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# The business of giving

W. F. Bynum

**The Circuit Riders: Rockefeller Money and the Rise of Modern Science.** By Gerald Jonas. W. W. Norton: 1989. Pp.430. \$22.95.

THERE are some people who would argue that it is at least as difficult to give away money wisely as it is to make it in the first place. Most scientists neither make nor give away large sums, but few experimentalists could work nowadays without somebody, from somewhere, providing them with a good deal more than their monthly pay cheques. Equipment, materials and technical assistance are expensive, and the business of grantsmanship looms larger for academic scientists than for their colleagues in arts or social sciences faculties.

Religious, educational, social, moral and health-care philanthropy have been around for centuries. Scientific philanthropy is barely a century old, but modern science has been indelibly shaped by it. The line between philanthropy and patronage is a fine one, but if we identify the first with the private sector and the second with the state, or public sector, some significant differences between the United States and Britain emerge. The relatively early establishment of the Research Councils — beginning with the forerunner of the Medical Research Council before the First World War — ensured that the state played a dominant role in research support in Britain. This is still the case even if Thatcherite policies have made the private research charities more important (the annual budget of the Wellcome Trust, Britain's largest private medical research charity, is now almost as large as that of the Medical Research Council). In the United States, however, before the Second World War the federal government's commitment to scientific and medical research was dwarfed by that of the big private foundations bearing the names of the industrialists (or robber barons) whose fortunes had created them. In the past few decades, the budget of the National Institutes of Health has put into the shade even that of the largest American foundations, which in turn are gigantic when compared with the best that Britain can muster.

The foundations, endowed by self-made men such as Andrew Carnegie and John D. Rockefeller Sr, marked by their very magnitude a new epoch in the history of philanthropy. They were in the wholesale end of the charity business and their massive scale meant that their founders could not possibly personally oversee



A couple of swells — the J. D. Rockefellers senior and junior in 1921.

giving away every penny of the largesse. Carnegie tried hard to do so, gradually withdrawing and eventually retiring from business (his was steel) to devote himself totally to his various philanthropic concerns, including education, public libraries and world peace. But even he had to rely on advisors, especially after scientific research was added to his charitable portfolio. Rockefeller (his money came from oil) was always more aloof, confining himself mostly to the getting of wealth and allowing a new breed of individuals — who called themselves 'philanthropoids' — to oversee its distribution.

Philanthropoids are people who earn their living by giving away someone else's money, and it was they who insisted that their jobs were not necessarily easy; the biography of Alan Gregg, one of Rockefeller's philanthropoids, was entitled *The Difficult Art of Giving*. Rockefeller's philanthropic employees were convinced, however, that he had a moral duty to give away a substantial proportion of his enormous fortune: "You must distribute it faster than it grows! If you do not, it will crush you and your children and your children's children", Frederick Gates cautioned him in 1905. Rockefeller's descendants have not wanted for the odd copper to spend on themselves, but the founder of the clan did establish a pattern of giving which suggests he heeded the advice of Gates and his other advisors. Carnegie and Rockefeller gave away \$850 million during their lifetimes, a sum which must be put into the buying-power perspective of their deaths in 1919 and 1939 respectively. (Their combined ages of 181

remind us that they had plenty of time to give it away, and also that they did not seem to suffer from any diseases of guilt.)

Gerald Jonas's volume assesses, as its subtitle makes clear, the effect of Rockefeller's legacy on modern science. Its sideways glances at Carnegie are incidental, but appropriate, because the two magnates saw themselves as philanthropic rivals. Until relatively late in his life, Carnegie devoted much of his money to fairly traditional 'missionary' activities: trying to make ordinary individuals healthier, or happier, or better educated through things like schools and libraries. There was a missionary side to Rockefeller's endeavours as well: reconstruction during and after the First World War, or the campaign to eradicate hookworm in the American South. Missionary work simply applied existing knowledge or helped rebuild what war had destroyed.

Gradually, Rockefeller became convinced that the motto of his foundation ("Towards the betterment of mankind") was best achieved through the creation of new knowledge, above all in science and medicine. Ironies abound: it was Gates, a Baptist minister, who read William Osler's *Principles and Practice of Medicine*, with its realistic, pessimistic account of medical therapy, and persuaded Rockefeller that medical research was a good thing. One result was the Rockefeller Institute (now Rockefeller University). When Rockefeller established his institute in 1901, his personal medical care was being overseen by a homoeopathist. Rockefeller shared Gates's Baptist faith: one result was a massive channelling of money which transformed an insignificant Baptist establishment called the University of Chicago into a great secular research-orientated university.

The foundation's emphasis on scientific and medical research inevitably meant more of Rockefeller's philanthropoids were scientists or doctors themselves, or men such as the philosopher Wickliffe Rose who had absorbed the gospel of science. Warren Weaver, a physicist, was instrumental in Rockefeller support for a discipline which Weaver himself first called "molecular biology", and the bacteriologist Selskar M. (Mike) Gunn kept Rockefeller's European operations going for a crucial decade.

In the nineteenth century the internationalism of science had been effectively espoused, and Rockefeller's philanthropoids shared this ethos. This meant creating schools of public health throughout the world, or giving money for academic medicine in Britain and physics research in Germany, or helping scientific refugees from Nazi Germany, or supporting Howard Florey's penicillin work, were as much in their brief as the considerable sums which stayed behind for the emerging scientific community in the United States. ▶

Except for a couple of summary chapters, Jonas's account ends just after the Second World War. It is thus confined to the 'circuit riding' period of Rockefeller philanthropy, before the modern peer-review system emerged. Like the frontier Methodist preachers riding their circuits, Rockefeller's philanthropoids travelled incessantly in search of worthy recipients. Officials at the top of the Rockefeller hierarchy had enormous power which, on balance, they seem to have used reasonably. Jonas (a staff writer for *The New Yorker*) does not attempt to tell the whole story. There is little on the Rockefeller

Institute itself, or on the endowment of the schools of public health, and rather too much on Florey and penicillin. But he is good on the gradual shift from bricks and mortar philanthropy to more support for individual scientists, on the development of informal networks of advisors, and on the personal contributions of key philanthropoids. Jonas gives us an apposite portrait of modern science in the making, but still not fully made. □

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## Mad for money

Daniel S. Greenberg

**Unguided Missiles: How America Buys Its Weapons.** By Fen Hampson. W.W. Norton: 1989. Pp.348. \$19.95.

THE military establishment in the United States is often depicted as a rogue enterprise that thrives against the will of the body politic. President Dwight Eisenhower suggested that conception in his valedictory warning against the "military-industrial complex". Numerous works, academic and journalistic, have taken up the theme, exposing the wily collusions of the Pentagon and its political and industrial collaborators.

Hampson, a professor of international relations at Carleton University, Ottawa, carries on the tradition by applying economic analysis and game theory to a half-dozen of the military's grandest research and procurement extravaganzas — the B-1 bomber, the Trident missile system, the MX missile, the air-launched cruise missile, the Strategic Defense Initiative and the Abrams tank (covered in a chapter by a colleague, N. Swales). Referring to and quoting heavily from numerous works by others, Hampson observes that these systems have survived years — even decades — of political opposition, premature obsolescence and cost increases vastly beyond the original estimates. Except in rare instances, weapons systems that have passed the research stage have proven to be invulnerable to termination, whether for political or technical reasons. Why?

The answer, says Hampson, is that the military services compete for budgets up to a point, but their rivalries are "marked by 'competitive cooperative' behavior akin to that noted by economists in oligopolistic settings and by game theorists in iterated or repetitive games" (p. 301). Specifically, the sacred systems of each service — the Navy's carriers, the Army's tanks, and the Air Force's missiles and manned aircraft — are out of bounds for

serious challenge by the others.

So much for the collusion of the military services. What of their presumed political masters in Congress? "Congress almost never addresses the fundamental question 'Should we fund this program?' Rather, the question is 'How much?' Once we understand the logic of this process it becomes easier to see why weapon programs rarely die at the hands of Congress" (p. 47). One reason they don't die is the high 'pork' value of defence spending, as typified by the distribution of B-1 bomber subcontracts to 48 states. When a challenge to the programme's budget developed, the Air Force and its main contractor, Rockwell, roamed Capitol

major rationale for the MX, a missile system that survived 13 years of difficult gestation. Advocates of arms control feared it as a provocative first-strike weapon. And no satisfactory means of moving it about was ever devised. As a last resort, the MX was based in stationary silos originally built for Minuteman missiles. Hampson notes that Carter tolerated the MX's survival "to appease Congressional hardliners who worried that arms control would lull the United States into a false sense of security". He adds that "Worries about ICBM [intercontinental ballistic missile] vulnerability were all but swept under the rug of political expediency" (p. 143).

From the case studies, Hampson concludes that the military establishment, industrialists and politicians all view the defence budget as a bountiful resource that can accommodate all claimants if none is too greedy. The bargaining process, he explains, "assures rewards or payoffs to all parties from cooperative behavior. A program will therefore not get all of the funds and resources its sponsors want, but, by the same token, it will rarely be killed" (p. 303).

By combining the rogue thesis with game theory and economic analysis, Hampson thus provides useful insights into *how* the system works. Unfortunately, only passing attention is paid



Pork on the wing — the US Air Force B-1 bomber at Farnborough, Britain in 1982.

Hill, with lists of every contract cross-referenced by state, town and Congressman.

And the White House? During eight years of Reagan, it never met a major weapons system it didn't like. But even before Reagan, the champions of big weapons systems rarely encountered effective political opposition. Jimmy Carter, for example, banned the start of production on the B-1. But, as Hampson reports, Carter later lamented that "the enormous B-1 lobbying octopus was still alive and writhing. It would live to fight again after I left the White House" (p.163).

Again and again, the weapons champions have overcome political resistance and even technological good sense to field their systems. Mobility, as a safeguard against a sudden Soviet strike, was a

to a more important and little-explored issue: why is the system tolerated, when, demonstrably, its extravagance and technological obtuseness detract from national security and result in the squandering of resources? What is the fertile ground on which this venerable military-political-industrial complex has thrived for 40 years?

The answer, of course, is the Soviet threat, surely real, but repeatedly manipulated and misrepresented by the 'weaponers' to undermine opposition and to create a climate of opinion favourable to any weapons system — no matter how foolhardy and expensive. Hampson observes that "Little doubt exists that intelligence and threat assessments are highly politicized assets in weapons programs" (p. 287). He notes, too, that "As the level of resources required by a

Associated Press



program grows, its sponsors may project the external threat more vividly and characterize it in terms of greater rather than less certainty" (p. 286).

There's paydirt in those themes — far more than in the exhausted thesis of inter-service and political backscratching as the explanation for the success of the weapons economy. For decades, US political consciousness has been steeped in fears of aggressive Soviet military intentions. Reagan drew applause from across the political spectrum when he decreed that defence spending must grow while all other government spending would be reduced. During his two terms in office, the military share of federal research spending rose from 50 to 70 per cent of the total — there were virtually no dissenting voices. In electoral politics, an unrebutted allegation of weakness on defence is almost invariably fatal. Just before taking office, the new Secretary of Defense, Dick Cheney, warned that it would be "a grave

mistake" to cut defence spending.

Hampson concludes that the "weapons acquisition and budgetary process must be restructured so that it becomes more of a zero-sum game" (p. 279). Towards this goal, he recommends stricter Congressional oversight, clearer budget presentations, and various other tinkering with the political and organizational underpinnings of the system. All have been suggested before. At one time or another, some have been put into practice, only to fail in their intended objective of rationalizing the design and purchase of weapons. That failure does not arise from defective bureaucratic arrangements. Rather, it comes from a distorted world view that insists that a menace is at the gates and nothing is too costly, or too mad, in the quest for security. □

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## Standard bearers

Philip Gummert

**The Evaluation of Scientific Research.** Ciba Foundation, chairman Sir David Phillips. Wiley: 1989. Pp.284. £32.50, \$57.95.

ONE of the first papers that I submitted for publication was returned by the editor, saying that he would take it if I removed a section that he judged to be of no interest. Being keen to get into print, I complied. Later, I decided that the excised section was still worth publishing. I sent it, duly smartened up, to another editor, who accepted it. At a stroke my productivity, as measured by publications, had apparently shot up.

Such an experience induces a healthy scepticism about performance indicators for research. Current talk in academic common rooms about 'citation rings' and 'smallest publishable units' only adds to the scepticism. Yet performance indicators, and evaluations of scientific research based upon them, are unquestionably here to stay. We have to learn to live with them, which means learning what can sensibly be used, for what purposes, and within what limits.

The Ciba Foundation therefore did valuable service by holding, in June 1988, an international conference of experts in evaluation to take stock of the state of the art. This book is the final product of the meeting. It is made up of 14 short papers, an introduction and summing up by Sir David Phillips, chairman of the UK Advisory Board for the Research Councils, and the text of the discussions. What emerges is a variegated blend of techniques,

methodological issues and case studies. Clearly, evaluations now come in all shapes and sizes. Some are concerned with evaluating national performance in science and technology; some with institutions, such as the British technological universities; some with programmes, such as the Alvey fifth-generation computer programme; and some with research groups. Others explore the operations of peer review systems; the impact of different modes of research funding; and the links between science and technology. One paper even attempts to evaluate the evaluators — surely a growth subject.

Underlying the whole discussion is a tension between those who are confident of the value of quantitative evaluative methods, hedged with caveats about the need for sensitive and skilled handling, and those who express concern that what can be quantified are only epiphenomena. Thus, although one contributor dismisses the long-standing debates on the meaning of citations as "rather arid" (p.13), others argue that indicators and evaluative techniques can only be worthwhile within the framework of a clearer understanding of the dynamics of science and technology, and their interaction with the economy.

To some extent, as is pointed out in the book, this is a debate between pragmatic science policy analysts and more theoretically inclined historians and sociologists of science. But it would be wrong to 'sociologize' away the debate this easily. For example, the book contains ample evidence of disagreements among even the most pragmatic participants over the acceptability of various bibliometric procedures, even down to such questions as how to count multi-authored papers, or how to define fields of science. These are not purely technical questions; their reso-

lution depends in part upon what one believes to be the importance of collaboration for the advance of science, or upon one's view of the relations between fields.

Indeed, disagreements over such points raise the question of what precisely is the evaluator's art. The contributors to this volume offer various answers. One sees himself as a guerilla rather than a regular soldier, though it is surely unusual for guerillas to be paid by the established government. Others discuss whether their role model is management consultancy, medicine, the law, designers of expert systems or writers of spy novels. The claim by one contributor, that those attending the conference were "a community of technicians in the business of evaluation", earned a sharp riposte from another participant who said that those present who felt themselves to be part of the scientific community were more concerned with understanding how science works, and what the scope might be for any kind of policy influence on the production of scientific knowledge (p.88).

Such differences of view also raise the issue of how professional standards are to be upheld among a group of analysts with growing influence in the corridors of power. There are disturbing references in the book to evaluators being sent back to "improve the picture" (p.28), and to the difficulties of maintaining integrity in the close relationship that, for a successful evaluation, exists between evaluator and customer. Statements about the importance of remaining outside political debate sit awkwardly against a long-established literature on the impossibility of scientific advisors acting apolitically. Most participants also seemed curiously unaware that, as one of their number pointed out (p.29), the activity of programme evaluation is not new, having been done on a large scale in social policy for 30 years, and that many of these issues have been addressed before.

One suggestion that emerged was for a code of ethics for evaluators to be laid down. Another was that evaluators should seek professional legitimation from within the field of science and technology studies, which could provide an intellectual critique of the meaning of indicators and techniques. Given the scale of commitment to evaluation of scientific research, these matters ought urgently to be addressed. As Sir David Phillips remarked, it may be that, like the proverbial drunk looking for a lost wallet, we are searching under a street lamp simply because that is where it is light — particularly the light provided by the *Science Citation Index*. Whether we are learning much about real scientific performance, however that may be defined, is a serious question that this book does much to air. □

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# Independence of mind

Michael Berry

**Journey Into Light: Life and Science of C.V. Raman.** By G. Venkataraman. *Indian Academy of Sciences*: 1988. Pp. 570. Distributed in Britain and the United States by Oxford University Press, £22.50.

THE past two years have seen centenaries of the birth of several of India's most brilliant citizens: Nehru, founder of the modern state; Ramanujan, diviner of amazing mathematical formulae; and Raman, the prolific and original physicist whom this book celebrates. Raman's life and work were complex and many-sided, causing great difficulties of judgement and emphasis for any biographer. Venkataraman (who in spite of his name does not share with several other talented Indian scientists the distinction of being a relative of Raman) solves these problems by anchoring his story firmly in the science. He has produced a model of scientific biography, written with respect and affection for its subject but with a clear-eyed perception of his faults, in a relaxed and gently witty style.

Raman's work was on the physics of waves and overwhelmingly centred on optics. He is best known for the effect that bears his name, but that was not discovered until he was 40 years old, when he was already established internationally as an authority on a variety of subtle, classical interference and diffraction phenomena such as the colours of heated metals and layers of bubbles. He had also studied the production of musical tones. An interesting discovery was that Indian drums have skins whose thickness varies radially in such a way that the frequencies of the overtones are integer multiples of the fundamental, so that they sound more harmonious than their Western counterparts, whose skins are radially uniform and generate irrational overtones.

The Raman effect is the scattering of light with a change of frequency (that is, inelastic scattering); it occurs in liquids, solids and isolated molecules. The change in frequency occurs when the light exchanges energy with the internal vibration or rotation states of whatever scatters the light. Compared with the elastic, or Rayleigh, scattering, for which the incident and scattered beams have the same frequency, the Raman effect is weak, which is why it was not discovered earlier. Raman (with Krishnan) found it by deploying simple apparatus with great experimental skill. The discovery was announced in *Nature*. Venkataraman mentions anecdotal evidence that the paper was first rejected. New evidence confirms this: apparently there were two unfavourable referees, both Fellows of the Royal Society, who provoked in the

editor (Sir Richard Gregory) the opinion that any paper inspiring such vehement opposition must have something good in it and so should be published! The paper stimulated a great deal of immediate and continuing interest, not only by demonstrating a fundamental optical effect fully concordant with the (then) new quantum mechanics but also by providing a sensitive probe of the vibrational and (for molecules) rotational structure of matter.

Raman's creative work did not stop



Indian Academy of Sciences

Offering enlightenment — Raman on crystals. with the Raman effect. I was surprised to learn that he discovered or anticipated several phenomena usually regarded as having been found decades later by others. One of these is what is now called 'speckle'. This is the mottled appearance of coherent light scattered by static randomness such as grains of powder on a screen or irregularities on a rough painted wall. Nowadays speckle is a familiar accompaniment of images produced by lasers, but Raman (with Ramachandran) saw it in light from a mercury lamp filtered by a pinhole. He had a complete understanding of the phenomenon, 20 years before lasers were invented.

Another anticipation is of what are now called 'soft modes'. A soft mode in a solid is a vibration whose frequency vanishes as a critical temperature is approached. The vanishing indicates the weakening of the structure, which changes abruptly at the critical temperature. Raman (with Nedungadi), using Raman spectroscopy,

observed the decrease in a lattice vibration frequency of quartz and correctly understood its association with a structural phase transition.

A high point of Raman's work after the discovery of the Raman effect was his theory (with Nath) of the diffraction patterns produced by light traversing refractive-index corrugations made by irradiating a liquid with ultrasound. As Venkataraman puts it, in a chapter charmingly titled "Son et lumière",

Raman loved waves, and this problem had light waves as well as sound waves. What more could he ask for? . . . The full-fledged Raman-Nath theory was put to a stringent test only in 1963 . . . [and] is as good as one would want it to be, provided one takes the trouble of carefully solving the equations.

Recently I learned that this theory too is an anticipation: exactly the same equations have been rediscovered in the theory of free-electron lasers.

The failures of great scientists are often as interesting as their successes, and Raman had his share of failures. Perhaps the best known is his controversy with Born about the vibration spectrum of diamond. Because of a persistent mathematical misunderstanding of the dynamics of crystal lattices, he never accepted the fact that the vibration frequencies form a continuum. He predicted a discrete spectrum, on the basis of a 'quasi-molecular' theory of his own, and he carried out experiments that seemed to confirm it. As Venkataraman explains, Raman's theory, although wrong, picked out a particular subset of the vibrational modes, at which the spectrum has singularities that Born did not know about but whose existence was later demonstrated by Van Hove. His experiments had fairly low resolution and so he saw only the singularities, masquerading as sharp lines. In other words,

Raman was locally correct but globally wrong. The part he got right is an important part and reflects his native brilliance. He would not have erred the way he did had he been trained properly in quantum physics.

I long suspected that Raman never properly understood the connection between wave optics and geometrical optics. That suspicion is confirmed by the account here of his ideas about the mirage. By imagining the air above a hot surface as a stack of slabs of slightly different refractive indices, he convinced himself that refraction could never make a downward-sloping ray turn upwards, because a ray once horizontal would remain so. In other words, refraction could never simulate reflection. This is a misunderstanding of the law of refraction in a continuously varying medium; the same argument, applied to an obliquely fired projectile, would predict that it would never fall but would continue horizontally on reaching its greatest height. The mistake led him to think the mirage could be explained only



with a wave theory. He constructed such a theory, and it was correct, but rather than being an anticipation of later work, it was a rediscovery of a result published by Airy in 1838.

Raman's collaborator on the theory of the mirage was his talented nephew Pancharatnam, fated soon to die at a tragically early age. Venkataraman gives a detailed account of Pancharatnam's ideas on the interference of polarized light. Only now are we beginning to appreciate their originality and depth — for example his anticipation of the geometric phases now in fashion.

These and many other areas of physics are explained with elegance and clarity. The author is a theoretical physicist with a flair for simple and direct exposition. Sometimes more sophisticated mathematical treatments are merited, and these are given in separate sections so as not to interrupt the main narrative.

Raman seems to have been a difficult and obstinate man. His dealings with administrators and bureaucrats, men of narrow vision compared to him, were

stormy, and he made enemies among his colleagues. He became embroiled in controversy and resigned in rage, first from his chair in Calcutta and then from the directorship of the Indian Institute of Science in Bangalore. Finally he founded his own Raman Research Institute in Bangalore. That Institute continues today as a jewel in the crown of Indian science, a tribute to Raman and his successor Radhakrishnan. Venkataraman discusses these matters in great detail and with sensitivity. His book makes us appreciate how unusual Raman must have been to produce such an abundance of creative science, first in the stifling colonial atmosphere of British India, and then in the turbulent struggle for independence. □

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• Oxford University Press will also be the distributor of Raman's collected works, in six volumes and under the title *Scientific Papers of C. V. Raman*. The books will become available in July–August, price £25, \$49.95 per volume.

## Well does He?

Robert M. May

**Does God Play Dice? The Mathematics of Chaos.** By Ian Stewart. Basil Blackwell: 1989. Pp.317. £15. To be published in the United States in May, c. \$19.95.

READERS OF *Nature* will recognize Ian Stewart as the person whose News and Views articles continually demonstrate that it is indeed possible to convey the excitement — and the substance — of advances on the frontiers of pure mathematics to a wide audience. *Does God Play Dice?* is aimed even more widely, to the reading public at large.

The book opens with a far-reaching proposition (with which I agree). In earlier times, the world was seen as unpredictable, governed at best by capricious deities. Over the past few centuries, the newtonian revolution has given us the opposite view, with most things seeming to be predictable provided one has the right rules or equations and enough computing power. But we may be at the start of another swing of the pendulum, with the recognition that many simple and fully deterministic rules give essentially unpredictable or chaotic dynamical behaviour.

In his second and third chapters, Stewart sets the stage with succinct yet lucid accounts of the newtonian paradigm ("Equations for Everything") and of the view that chance or random behaviour derives from complexity ("The Laws of Error"). Poincaré ("The Last Universalist") is seen as bestriding a watershed. Refining

the newtonian tradition, he sought qualitative, geometrical ways of understanding the behaviour of systems of differential equations. In the process, he gave birth to the discipline of topology, and initiated the long transition from the classical mechanics of the nineteenth century (with its increasingly elegant solutions of those subsets of equations that could be solved elegantly) to the dynamical systems theory of today. As translated by Stewart, Poincaré recognized what are now called strange attractors in a simplified version of the three-body problem:

When one tries to depict the figure formed by these two curves and their infinity of intersections [one gets] a kind of net, web, or infinitely tight mesh; neither of the two curves can ever cross itself, but must fold back on itself in a very complex way in order to cross the links of the web infinitely many times. One is struck with the complexity of this figure that I am not even attempting to draw.

Stewart launches from this springboard into a lively account of contemporary work on chaos: Smale's formal analysis of strange attractors; the chaotic behaviour (and beautiful regularities) of the quadratic map and other first-order difference equations with 'one hump'; Lorenz's already-classic set of three differential equations as a metaphor for unpredictability in weather systems; the Voyager data and Wisdom's computations on the chaotic tumbling of Hyperion, a moon of Saturn; and much else.

Fractals, the Mandelbrot set and other fashionable topics are also covered, but are clearly tied to the central theme enunciated in the opening chapter. In particular, Stewart gives an excellent,

intuitive account of the relation between fractals and chaos (it is not widely appreciated that the geometrical distinction between smooth forms, such as circles and spheres, and rough forms, such as fractal objects, is formally akin to the distinction between the familiar attractors of classical dynamics and the strange attractors of chaos; a fractal dimension can be associated with any given strange attractor, characterizing qualitative features of the dynamics).

Stewart's book will inevitably be compared with James Gleick's deservedly successful *Chaos: Making a New Science* (Viking, 1987). Both books do a good job of presenting complicated material in a way that is engaging, accurate and accessible to the uninitiated. Both also convey the fascination of the subject. Gleick spends more time on personalities, and on the excitement of the chase. This, I think, may make his account a better read for the layperson, and means that it captures the existential, higgledy-piggledy way science really advances. Gleick's understandable affection for colourful personalities sometimes leads to discrepancies between his 'programme credits' and the perceptions of those in the discipline about who did what first, which has led to some rough reviews by professionals (for example that in *Nature* 330, 293; 1987).

Although racily written, with lots of staccato paragraphs such as "Simple. Elegant. Elusive." or just "No.", Stewart's book concentrates rather less on people and more on the science itself. Even so, its programme credits are not impeccable. The basic catalogue of the various periodic orbits for generically quadratic maps is attributed to Sharkovskii, whereas among the many independent discoverers I believe the neglected Myrberg comes first; the bifurcation diagram for this map was first published, and its properties of self-similarity on smaller and smaller scales noted, by Oster and myself in *American Naturalist* (110, 573-599; 1976), but here the diagram is as usual called the Feigenbaum tree, permitting a multilingual 'fig-tree' pun. Such attributions of priority matter only to the players. The play itself is what really counts and here Stewart is, not surprisingly, stronger than Gleick on the mathematics, particularly on how the various aspects of the subject fit together.

In a thought-provoking final chapter Stewart speculates upon the implications of our still-emerging understanding of chaos. Observing that "order can breed its own kind of chaos", he returns to the celebrated exchange between Einstein and Born, and suggests "The question is not so much *whether* God plays dice, but *how* God plays dice". □

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# When seeing is believing

Albert Van Helden

## Planets and Perception: Telescopic Views and Interpretations, 1609–1909.

By William Sheehan. University of Arizona Press: 1988. Pp. 324. \$35, £23.95.

*Planets and Perception* is a somewhat misleading title. Rather, Sheehan's book is specifically about the Martian canal episode, from its start with Schiaparelli in 1877 to its demise at the hands of Antoniadi in 1909.

By way of background, the first 50 or so pages take the reader from Galileo's first telescopic observations to the middle of the nineteenth century, with brief excursions into the debate about extraterrestrial life. The action proper starts with the favourable opposition of Mars of 1877, when Asaph Hall discovered the two Martian satellites and Giovanni Virginio Schiaparelli announced that the planet's surface was laced with *canali* (which in English became *canals* with all the implications of intelligent design). In this story, which has been told before, the main protagonists were Schiaparelli, the hydraulic

engineer turned astronomer with his penchant for sharp geometric delineation; the French astronomer and popularizer Camille Flammarion (who was fired by LeVerrier, the director of the Paris Observatory, for his speculations about extraterrestrial life); the Boston Brahmin Percival Lowell, whose mind was made up before he ever made a serious study of Mars; and the remarkable Eugene Marie Antoniadi, a native of Constantinople, who made an astronomical career in France and for many years held the position of director of the Mars section at the British Astronomical Association.

Sheehan is an amateur astronomer who makes his living as a psychiatrist. The combination of interests makes him ideally suited to deal with this complicated episode in the history of astronomy. After all, this is not merely a story about the march of telescopic progress or the history of an egregious error made by inferior scientists. It is as much about apertures, diffraction and atmospheric seeing as it is about rods and cones, hard-wired biases in

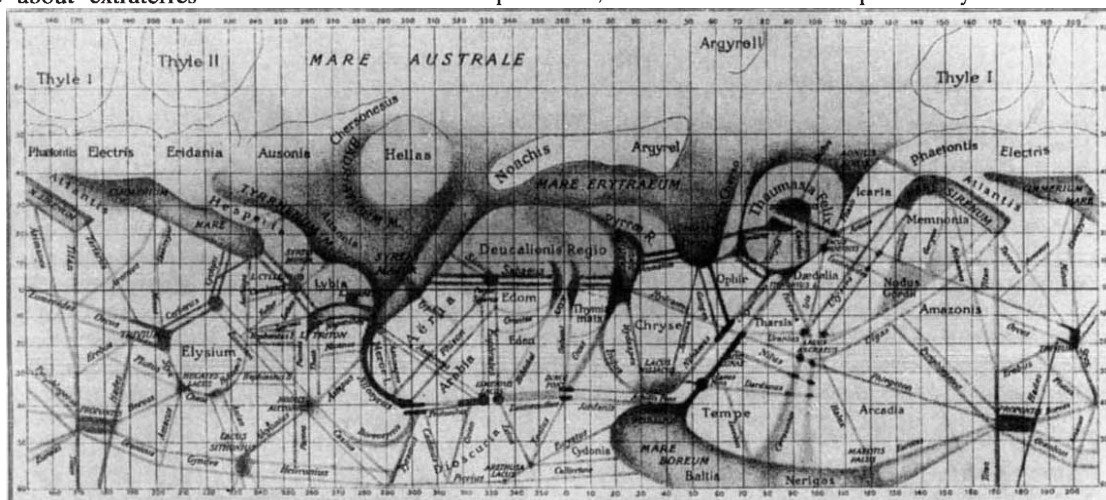
our perceptual apparatus, and the astronomer's professional and psychological background. Sheehan does a fine job of weaving all these strands together in a coherent and engaging account: his book is a good read.

The problem of the Martian canals is not so much that the observations were wrong. A number of talented observers with impeccable credentials saw these canals, and there is little point arguing that they could not have seen them because they do not exist. They knew that they saw them and they agreed with each other that they really did exist. Somehow the chronicler of this episode must explain how it is that virtually an entire generation of planetary astronomers could see vague and uneven shadings on Mars's surface as canals — not as something else, but as canals.

The explanation must begin with an account of the telescopes used, their

good account of how canals were produced. All this is incorporated in an engaging narrative.

Finally, however, we must come to terms also with the personality of the observer. This is most obvious in the case of Percival Lowell, who founded an observatory just to prove his hunch that there was intelligent life on Mars. Lowell was an autocrat of enormous energy and great charisma. When he turned his attention to astronomy it took him less than a year to certify the canals and to launch a veritable campaign — among scientists and the public — for the acceptance of life on Mars. Here Sheehan is cautious, as befits a professional who is aware of the strengths as well as the limitations of his field. But we are given tantalizing glimpses of Lowell's relationship with his father, his bouts of neurasthenia and his relationship with his secretary. Surely in this area lies a potentially fruitful field of



Lines of thought — Mars, 1890, from the *Opera de G.V. Schiaparelli*, Vol. 1.

resolution and the quality of the atmosphere in which they were used. In the late nineteenth century there was a great debate about the efficacy of very large telescopes in planetary observations that focused the attention of astronomers on the workings of the Earth's atmosphere. Although the issue was finally settled in favour of the larger instruments, for several decades there was widespread doubt that the largest apertures (up to 40 inches) were as effective as more modest apertures, and Percival Lowell himself almost always stopped down the aperture of his 24-inch refractor to 12 to 18 inches. Obviously this debate had crucial implications for the question of the canals. Sheehan explains this aspect very well indeed.

How a series of faint and irregular markings, perhaps too small to be seen individually, can be perceived as straight lines is a more complicated exercise that takes Sheehan into the field of experimental psychology. He is able to put what actually happens at the telescope in this psychological context and constructs a

future research for one with Sheehan's expertise. Can we delve further into the personality of Schiaparelli, who used his revelations about Mars in a quite machiavellian way to push the Italian authorities into funding a larger telescope while often in private expressing doubt about his discoveries? Is there more to be said concerning the personality of Edward Emerson Barnard who, using the 36-inch Lick refractor, had by 1894 shown to his own satisfaction that the canals did not exist but did not press the issue? What can be found out about the flamboyant Camille Flammarion?

In the end we are left with the question of how astronomers ever got things right before the space missions to the planets. Those working at the research front, from Galileo to Antoniadi and Dollfus, always operated at the very limits of the discriminating powers of their telescopes. Sometimes, as in Galileo's case, they were right even when their colleagues could not immediately see what they were able to discern. At other times, as in the case of Schiaparelli, they were wrong even



though their colleagues verified their observations. Here is the great enigma of the history of telescopic astronomy. We need to learn what it was about Galileo, Cassini or Herschel that made them right. But before we can find the answer to that question, we need to know how Schia-

parelli and many of his contemporaries could be so wrong. Sheehan's account goes a long way towards answering that question. □

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## On the move

Martin Rudwick

**Drifting Continents and Shifting Theories: The Modern Revolution in Geology and Scientific Change.** By H. E. LeGrand. Cambridge University Press: 1988. Pp. 313. Hbk £30, \$49.50; pbk £10.95, \$16.95.

THE image of 'revolution' has been an integral part of the self-understanding of the modern Earth sciences ever since the theory that is now termed plate tectonics gained wide acceptance in the 1960s. One of the first major post-war volumes to favour crustal mobilism, the symposium on *Continental Drift* edited by Runcorn, appeared in 1962, the same year as Kuhn's *Structure of Scientific Revolutions*. The enthusiastic adoption of the kuhnian language of paradigms and revolutions by many Earth scientists in the later 1960s was no coincidence. It reflected a widespread sense of living through a period of dramatic conceptual change, which promised to transcend earlier disciplinary divisions and to make a unified 'Earth science' an attainable goal. Kuhn's model of scientific change, itself representing a dramatic break with orthodox philosophy of science, seemed tailor-made for Earth scientists. The use of such philosophical models to interpret the origin and development of plate tectonic theory is thus a tradition that stems from within the science itself.

Hallam's *Revolution in the Earth Sciences*, which was published in 1973 almost before the dust had settled, was one of the first — and one of the best — of a distinctive genre of historico-philosophical analyses by practising Earth scientists. 'Practitioner histories' have their limitations, however, because the professionalism of their treatment of the technical issues is inevitably offset by a less than professional acquaintance with the wider issues that any general model of scientific change entails. 'Participant histories', such as Menard's delightful *Ocean of Truth* (1986), are also invaluable; but like any other primary source they need to be treated as raw material for historical interpretation, rather than as unproblematical accounts of 'how it actually was'.

Homer LeGrand's new treatment of the history of 'Drift' — as he usefully terms all theories of crustal mobilism — is different, in that the author's professional affil-

iations are with the burgeoning field of science studies. Whatever his book may lack in the way of a participant's vivid recall of events, or a practitioner's tacit feeling for the science, is amply compensated by its sophisticated and up-to-date treatment of the historical, sociological and philosophical issues. As the title implies, the book is concerned with shifts in theories as well as continents.

Readers who like their history of science penny-plain are well served by a beautifully clear and concise narrative. This is grounded in a substantial bibliography; it is illustrated by reproductions of significant diagrams; and it shows an admirable mastery of the technical issues. The narrative is far less detailed than, for example, Glen's *Road to Jaramillo* (1982), but it covers a far wider field, and it is much better balanced than any comparable account. The book avoids the 'precursoritis' that turns Alfred Wegener into a neglected prophet or a retrospective hero. It also avoids the provincialism of some other histories of plate tectonics, which distort the picture by concentrating on those who constructed the theory in the form that became orthodoxy in the 1970s.

An early chapter sketches the late-nineteenth-century background of high-level theoretical debate between 'permanentists' and 'contractionists'; the contrast in scientific style between Europeans and Americans, which became so striking when Drift theories were revived in the 1960s, was clearly apparent even at this early stage. Global theorizing was an established and respectable tradition in German-language geology, and LeGrand neatly summarizes the lively debate that Wegener's *Origin of Continents and Oceans* (1915) evoked as soon as the First World War was over, not only in continental Europe but also in Britain. Even more importantly, he documents the unbroken tradition that linked those debates of the 1920s with the revival of Drift theorizing in the 1950s. The very possibility of Drift was indeed rejected vehemently by most of the leaders of the North American scientific community during this period, as it continued to be by Soviet scientists even through the 1970s. But for the Europeans, and still more for geologists in the Southern Hemisphere, various forms of Drift, progressively improved from Wegener's original formulation, remained a live option, albeit a minority position, that was continually under review.

Having established that vital continuity,

LeGrand shows how the crucial new input from the physical scientists in the 1950s, namely in the technique of palaeomagnetism and the theory of polar wandering, created an increasingly favourable climate for Drift theorizing among land-based geologists and geophysicists. Contrary to subsequent myths, this dramatic revival in the fortunes of Drift theorizing took place *before* the burgeoning breed of ocean-going scientists began to apply their mass of new data to the interpretation of global tectonics. The rest, as journalists are fond of saying, is history. However, LeGrand continues his narrative with an account of the emergence of plate tectonics from pre-existing forms of Drift theory. Notwithstanding his own title, he interprets this convincingly as a story of continuous conceptual evolution rather than as a revolutionary break with the past. Finally he traces the precipitate conversion of the North American scientific community, and, to avoid too triumphalist a conclusion, notes continuing pockets of sceptical resistance elsewhere in the world.

Readers who want a tuppence-coloured version of this history get it, at no extra cost, in the form of what LeGrand terms "Voice-Overs" at the end of each chapter. These are brief commentaries on the foregoing narrative, reflecting on its compatibility — or more often, incompatibility — with the main theories of scientific change discussed among philosophers of science in the past quarter-century. The 'paradigms' of Kuhn and the 'research programmes' of Lakatos are given respectful consideration, but are rejected as incompatible with this particular example of scientific change. LeGrand has more sympathy with Laudan's model of 'research traditions', although in my opinion his narrative does not lend it much better support than the others. The 'interest model' of the Edinburgh school of science studies, with its emphasis on the constitutive role of the socio-economic context of scientific work, is effectively applied, for example to the interpretation of the rise of ocean-going Earth science in relation to the needs of the United States Navy in the era of the Cold War. But in my view, what LeGrand terms the 'internal struggle' model, now associated particularly with Latour and other French analysts of science, is easily the best supported by his narrative.

This is a book that deserves a wide readership among Earth scientists of all stripes, as well as among historians, sociologists and philosophers of science. It gives the best brief narrative there is of the origins of plate tectonic theory; and it combines that with a thought-provoking and undogmatic evaluation of the story as a notable episode of conceptual change in modern science. □

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